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Title

Chemical diversity and mode of action of natural products targeting lipids in the eukaryotic cell membrane

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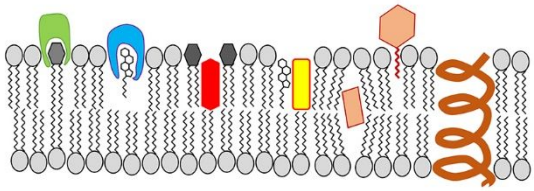
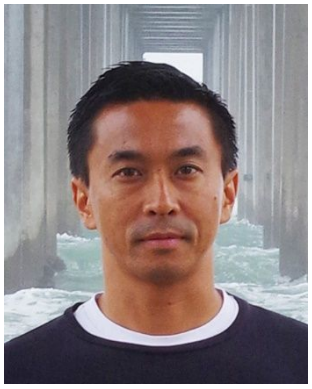


This review article focuses on natural products that bind to eukaryotic membrane lipids, and includes clinically important molecules and key research tools. The history of how their mechanism was unveiled, and how these natural products are used in research are also mentioned.

Author Profiles

Shinichi Nishimura received his PhD from The University of Tokyo in 2004 with Professors Nobuhiro Fusetani and Shigeki Matsunaga, where he identified the cellular target of a marine sponge-derived antitumor compound. He performed a postdoc with Dr. Minoru Yoshida at RIKEN, Japan, conducting chemical genomics for target ID of natural products (2004-2007). He hunted for bioactive natural products with Prof. Hideaki Kakeya at Kyoto University (2009-2018) and with Prof. William Finical at Scripps Institution of Oceanography, University of California San Diego (2016-2017). Since 2018, Dr. Nishimura has been a lecturer in the Department of Biotechnology at The University of Tokyo, where his research is focused on the molecular function and physiological role of natural products.



Nobuaki Matsumori received his PhD in 1997 under the supervision of Prof. Kazuo Tachibana at The University of Tokyo: his dissertation was on the development of J-based configuration analysis and its application to complicated natural products such as maitotoxin. After, he studied antibiotic biosynthesis as a postdoc with Prof. Sueharu Horinouchi at The University of Tokyo (1997–1999), he was appointed as an assistant professor (1999) and an associate professor (2010) at Osaka University, where he worked with Prof. Michio Murata. In 2000, he spent a year in the solid-state NMR laboratory of Prof. Robert G. Griffin at MIT. Since 2014, he has been a professor at Kyushu University. His current research interest is in elucidating the mechanisms of membrane-associated interactions such as inter-lipid, lipid-MP, drug-lipid, and drug-MP interactions.

Abstract

Nature furnishes bioactive compounds (natural products) with complex chemical structures, yet with simple, sophisticated molecular mechanisms. When natural products exhibit their activities in cells or bodies, they first have to bind or react with a target molecule in/on the cell. The cell membrane is a major target for bioactive compounds. Recently, our understanding of the molecular mechanism of interactions between natural products and membrane lipids progressed with the aid of newly-developed analytical methods. New technology reconnects old compounds with membrane lipids, while new membrane-targeting molecules are being discovered through the screening for antimicrobial potential of natural products. This review article focuses on natural products that bind to eukaryotic membrane lipids, and includes clinically important molecules and key research tools. The chemical diversity of membrane-targeting natural products and the molecular basis of lipid recognition are described. The history of how their mechanism was unveiled, and how these natural products are used in research are also mentioned.

Sections

1. Introduction

Natural products occupy wide parts of the chemical space and continue to open up unbeknown chemistry spaces. They often show potent biological activities with high specificity, unveiling molecular secrets of life and providing pharmacological benefits.[1-3] Any bioactive compounds with characteristic biological effects have specific cellular targets. Specific binding between natural products and cellular receptors is always enabled by precisely designed molecular recognition. One representative are the immunosuppressant molecules produced by microbes: FK506, cyclosporine and rapamycin.[4-5] They show different, complex chemical structures but target the same cellular receptors with high specificity and affinity. In many cases, compounds derived from natural products do not exceed the potency of the parent compound. Sophisticated molecular recognition is not limited to protein binding compounds, and can be found in compounds recognizing membrane lipids.

Amphiphilicity, the property of a chemical to have both polar and non-polar moieties, is a common physical property of natural products. Bacteria produce biosurfactants, for example, surfactin, a lipopeptide produced by *Bacillus subtilis*.[6] These surface-active agents disrupt membranes, causing antimicrobial and hemolytic activities, while they increase the cell motility and influence cell differentiation of the bacteria.[7] Marine invertebrates and plants contain saponins, which often show high sterol-binding activity, leading to antifungal and hemolytic activity.[8-11] Saponins are generally considered to be defense compounds against predators. In the marine environment, saponins of sea cucumbers were shown to work as kairomones that attract a symbiotic crab.[12] Amphotericin B, produced by streptomycetes, exhibits potent antifungal activity by binding ergosterol, and is a commonly used antifungal drug. It is likely that membrane-binding compounds are widely spread in our living world. We can expect that novel compounds with unique chemical structures and membrane-binding properties remain hidden in the expanding chemical space of natural products.[13]

In the past three decades, methods for identifying target proteins of biologically active small molecules have been developed, mostly based on physical interactions.[14-15] In these methods, compounds are modified and binding proteins are fished from a cell lysate, or pools of proteins displayed on phage.[16] However, compound profiling based on chemical genetic interactions or pattern analysis of cell morphology or the proteome have been developed for systematic analyses of modes of action of bioactive molecules whose targets can be proteinous or non-proteinous.[17-19] Non-proteinous molecules contain nucleic acids, glycan, ions and lipids. In this century, newly-developed chemical genomic methods have identified new classes of natural products targeting membrane lipids.[20-

22]

In this article, we first present a general introduction of the lipid structure and function, how we can investigate the lipid function, and druggability of membrane lipids. Then we review natural products that have specific lipid targets. Here, we classify lipid-targeting natural products into three groups: type A molecules recognize lipids through vertical interactions; type B molecules bind to lipids through lateral interactions; type C molecules are distributed to the lipid membrane owing to its amphiphilicity or hydrophobicity but show little lipid specificity. This review focuses on natural products that target the mammalian cell membrane and are categorized as type A or B. How their target lipid was identified, how their binding modes were investigated, and how they contribute to the scientific knowledge are included. Readers interested in learning more about natural products being categorized into type C or targeting the bacterial cell membrane are referred to previous reviews.[23-26]

2. Structure and functional analysis of lipids in the cellular membrane

Major lipids in the eukaryotic cell membrane include glycerophospholipids, sphingolipids and sterols (Fig. 1).[27-28] Lipids have a high structural diversity with an estimated 180,000 different species.[29] In the budding yeast *Saccharomyces cerevisiae*, one of the simplest eukaryotic model organisms, the size of the lipidome was estimated to be around 300.[30] Minor structural variations contribute to the large number of lipid species, e.g. position of unsaturation, methyl branching pattern, and sites of hydroxylation in acyl/hydrocarbon chains. Lipidomics analysis based on mass spectrometry and newly developed analytical methods are enabling researchers to grasp the whole view of lipid species.[31-32]

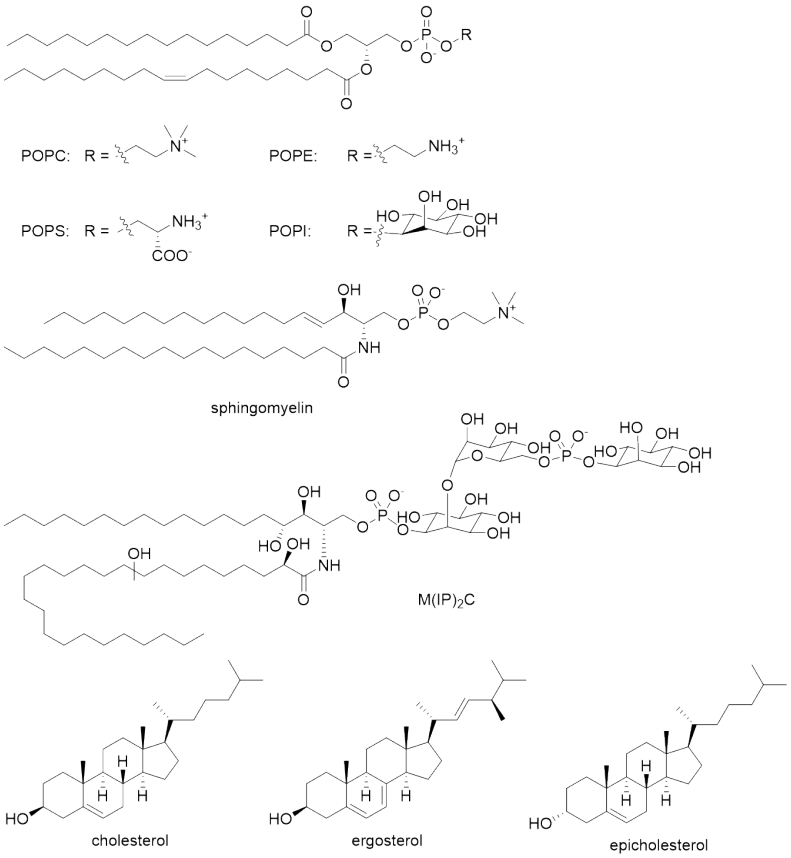


Fig. 1 Chemical structures of membrane lipids described in this article. Four glycerophospholipids, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI) are shown. The acyl groups attached are palmitoyl and oleoyl (PO). Sphingomyelin and mannosyl-diinositolphosphoceramide ($M(IP)_2C$) are major sphingolipids in human and yeast, respectively. The position of the hydroxylation in the very long chain fatty acid of $M(IP)_2C$ remains to be determined.[33] Three sterols are shown: cholesterol in humans,

ergosterol in fungi, and epicholesterol that has a 3α stereochemistry and is often used in sterol-binding experiments.

Lipids play multiple roles in the cell membrane: the lipid bilayer is a barrier between the inside and outside of the cell and forms intracellular compartments; lipids regulate physical properties of the membrane such as fluidity, curvature and surface charge; lipids regulate the activity of membrane proteins; lipids provide storage for carbon sources, energy and secondary messengers. Function of membrane lipids can be investigated by observing the phenotype of mutant cells lacking lipid biosynthetic genes.[27] For example, in the budding yeast *Saccharomyces cerevisiae*, uptake of tryptophan is reduced in an ergosterol mutant cells lacking the *ERG6* gene. Erg6 protein is a *C*-methyltransferase, converting zymosterol to fecosterol in the ergosterol biosynthetic pathway (Fig. 2A). Umebayashi and Nakano demonstrated that Tat2, a high affinity tryptophan permease, was not sorted to the plasma membrane correctly, but missorted to the vacuole in the ergosterol mutant, thus showing a defect in tryptophan uptake. This suggested that sterols regulate the protein sorting to the plasma membrane through organizing lipid rafts.[34] The physiological and molecular function of glycosyl inositol phosphorylceramides (GIPCs) in a plant *Arabidopsis thaliana* was unveiled by classical genetics.[35-36] *moca1* mutant plants, which were identified as hypersensitive to salt stress, had a mutation in a gene encoding inositol phosphorylceramide glucuronosyltransferase 1 (IPUT1). IPUT1 transfers a glucuronic acid (GlcA) residue from UDP–GlcA to inositol phosphorylceramide (IPC), which is followed by further glycosylation to synthesize GIPCs (Fig. 2B). Genetic analysis coupled with *in vitro* physical analyses demonstrated that GIPCs on the cell surface senses Na^+ ions in a high salt condition, which then regulates gating of Ca^{2+} influx channels into plant cells.[35]

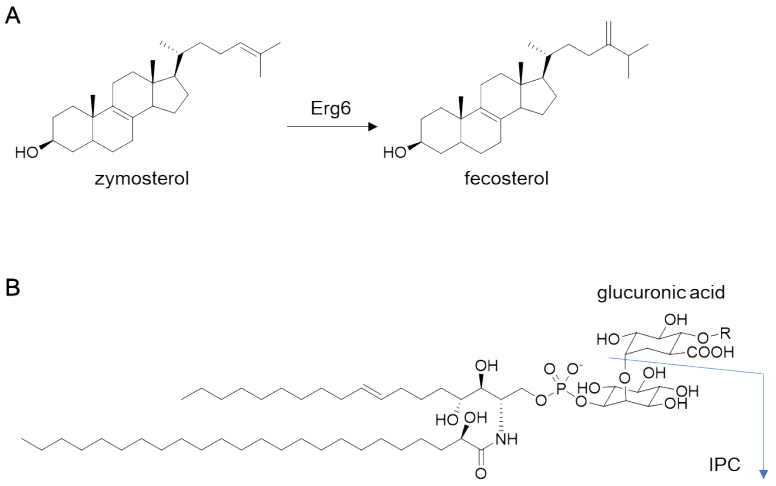


Fig. 2 Enzymes involved in lipid metabolism and their functions. (A) Erg6 is a *C*-methyl transferase converting zymosterol to fecosterol. In yeast, cells lacking this enzyme confer resistance to natural products targeting ergosterol. (B) Chemical structure of GIPC in plants is shown. IPUT1 catalyzes transferring glucuronic acid to IPC. This reaction is followed by further glycosylation to conjugate hexose at position 4 of glucuronic acid.[28, 36]

One thing we have to consider when the function of a membrane lipid is examined genetically is the metabolic enzymes in the downstream pathway. In the case of yeast cells lacking the *ERG6* gene, ergosterol is not synthesized but the substrate of Erg6 enzyme, zymosterol, is metabolized by downstream sterol biosynthetic enzymes to produce several species of sterols.[37] To avoid such a complex situation, exogenous molecules that directly bind to specific membrane lipids can be used. Methyl-ß-cyclodextrin is widely used to investigate the function of membrane cholesterol by removing cholesterol.[38] Bioactive natural products, such as cinnamycin and theonellamide A, are also useful: changes in the cellular morphology by these molecules can potentially unveil roles of specific lipids at specific subcellular localizations (see section 5.3 and 5.5, respectively).

Many hundreds to thousands of lipid species are distributed in the body, organs, and cells in specific manners. Subcellular localization of lipids is also tightly regulated and change with the cellular condition.[39-42] For example, phosphatidylserine (PS) is usually localized at the inner leaflet of the cell membrane, but exposed to the cell surface during apoptosis, which is recognized by phagocytes to be engulfed.[43] Abnormal localization is often observed under disease conditions, e.g. cholesterol and glycosphingolipids heavily accumulate in lysosomes of Niemann–Pick type C disease

tissues.[44-45] At a molecular level, lipid molecules and membrane proteins cluster with each other to regulate their molecular functions, which is called lipid raft.[46-47]

To analyze the cellular localization of membrane lipids, we have to use (hemi-)synthetic molecules and proteins: (A) labeled lipids; (B) lipid-binding proteins fused with a tag sequence; and (C) lipid-targeting natural products, which are chemically tagged (Fig. 3). To label glycerophospholipids, a reporter group, such as fluorescent function or alkyne, is attached to the head group or the alkyl chain (Fig. 3A).[48] In the case of sterols, the 3ß-hydroxy group can be conjugated with a reporter group, or a fluorescent group can be attached to the side chain.[49-51] Intrinsic fluorescent sterols such as cholestatrienol are also useful.[52] The disadvantage of these lipid tools is the possible loss of the physical property of the original lipid. Careful analysis of their physical properties is requisite. A small tag is usually preferred to avoid interference. Stable isotope labeling observable in imaging MS and a diyne tag detectable by Raman imaging were installed into sphingolipids to analyze lipid assemblages in cells and *in vitro*.[53-54] Next, lipid-binding protein toxins are useful lipid detectors. Some examples are derivatives of perfringolysin O, a thiol-activated cytolysin produced by *Clostridium perfringens*, which specifically binds to cholesterol, and lysenin, purified from coelomic fluid of the earthworm *Eisenia foetida*, which binds to sphingomyelin (Fig. 3B).[49, 55-57] Lipid binding domains found in membrane-localized proteins are also often used. These protein tools can be expressed from plasmids or DNA sequences integrated into the genomic DNA, by which intracellular distribution of the target lipids can be visualized. This strategy is unique because lipid-detecting probes usually do not penetrate into cells and only stain the cell surface in living cells. Finally, lipid-binding bioactive molecules can be used after installing a reporter group, such as biotin or fluorescent functionalities (Fig. 3C). Biotinylated cinnamycin is used for detecting PE, while fluorescently-labeled thoenellamides are used for detecting sterols. These will be mentioned in sections 5.3 and 5.5, respectively. The most widely-used chemical probe might be filipin, which shows intrinsic fluorescence when it binds to membrane sterols (for its chemical structure, see section 5.1).[58] However this compound induces membrane lysis after binding to membrane sterols and its photostability is low, thus is not compatible for analyzing cellular function of sterols.[59] The mechanism for the molecular recognition between sterols and filipin is not known despite its routine use in the cell biology field.

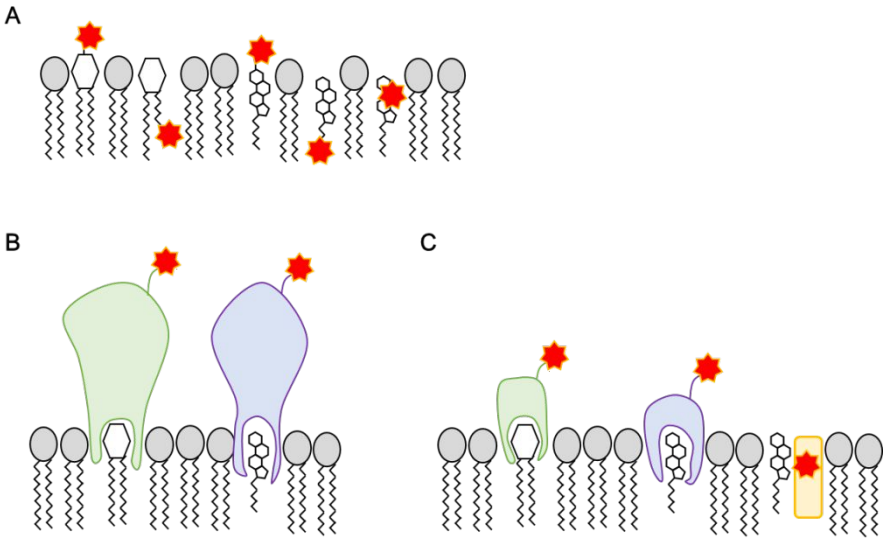


Fig. 3 Tools for visualizing membrane lipids. (A) Chemically labeled lipids can be used to report their cellular localization. Reporter functionalities can be installed into either the head group or the acyl group of the phospholipids (left two). In the case of sterols, the 3ß-hydroxy group or the side chain can be modified to be conjugated with reporter groups (central two). Intrinsically fluorescent sterol derivatives such as cholestatrienol are also useful (right). (B) Lipid-binding protein toxins or protein domains can be chemically or genetically tagged to detect specific lipids. (C) Natural products, binding to phospholipids or sterols, are modified to become fluorescent probes. These chemical probes are smaller and biologically more stable than protein tools. Filipin, which shows fluorescence when bound to sterols, has been used routinely (right), although where filipin resides in the bilayer is not known.

3. Membrane lipid as a drug target

The cell membrane is a major target of antimicrobial agents.[23] In the case of antibacterial compounds, daptomycin is one of the latest agents to be clinically approved, and is used to treat severe infections with Gram-positive bacteria (Fig. 4).[60] This cyclic lipopeptide is produced by a soil bacterium *Streptomyces roseosporus*. Daptomycin has been shown to permeabilize bacterial membranes and its effect is correlated with the level of phosphatidylglycerol (PG), although its molecular mode of action remains incompletely understood.[61] Recently, a promising new antibiotic lysocin E was isolated from the supernatant of *Lysobacter* sp. RH2180-5 using a silkworm infection model.[62] Lysocin E was shown to target menaquinone, an electron carrier important for aerobic synthesis of ATP, with a stoichiometry of 1:1 (Fig. 4). This compound also binds to lipid II, a major precursor of the bacterial cell wall, with an antibiotic-to-substrate binding stoichiometry of 2:1.[63]

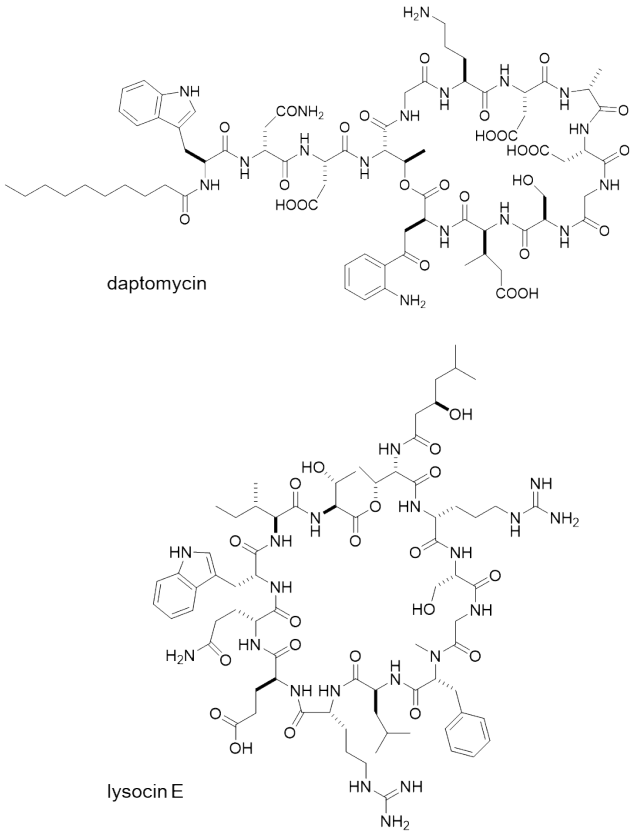


Fig. 4 Chemical structures of membrane-targeting antibacterial natural products.

Eukaryotic membrane lipid is also the drug target. Ergosterol is targeted by antifungal agents,[64-65] and cholesterol biosynthesis is the target of drugs for treating

hyperlipidemia.[66] Azole and allylamine drugs inhibit biosynthesis of ergosterol in fungi, while statins inhibit HMG-CoA reductase, thus reducing de novo synthesis of cholesterol. Ergosterol is directly targeted by polyene antifungals, such as amphotericin B, nystatin, and pimaricin (see **Section 5.1**).[67] Holotoxin, a saponin from a sea cucumber *Stichopus japonicus*, is part of the formulation of a drug used to treat athlete's foot (see **Section 4**).[68] In general, saponins are thought to target membrane sterols. In contrast to targeting intracellular enzymes for inhibiting ergosterol biosynthesis, direct targeting of ergosterol by amphotericin B rarely generates drug resistant strains clinically.[69] AmB-resistant mutants are hypersensitive to oxidative stress, elevated temperatures, and killing by neutrophils. They also have defects in filamentation and tissue invasion, thus being avirulent.[70-71]

When a protein is a promising target for combatting or curing diseases, high throughput screening for the protein ligand, based on the inhibitory activity of the enzymatic activity (if that is an enzyme) or physical interactions, is performed. By using the structural information obtained by NMR or X-ray crystallography, or by homology modeling, *in silico* docking simulation would suggest candidates of ligands after screening millions of compounds. However, screening for lipid-binding molecules is limited, although lipids are also promising drug targets.[72-73] In addition to sterols as described in the previous paragraph, fungal sphingolipids have different chemical structures from those of their human counterparts (Fig. 1).[28] PS and PE are specifically exposed on the tumor vascular endothelium.[74-76] Novel screening methods will enable identification of compounds that target these lipids.

It should be noted that natural products occupy diverse portions of the chemical space, especially with regard to their mass distribution and have an advantage for such screening because most membrane-binding compounds, including pharmaceutical drugs, have a molecular weight of > 500 Da (Fig. 5). This is incompatible with the rule of 5, which can be used to predict the drug absorption and permeation.[77] The recent finding of lysocin E (molecular weight: 1618 Da) with potent antibacterial activity and a new mechanism of action supports the idea that there are still large parts of the natural products chemical space that have to be discovered,[62] and its biological potential largely remains to be explored.

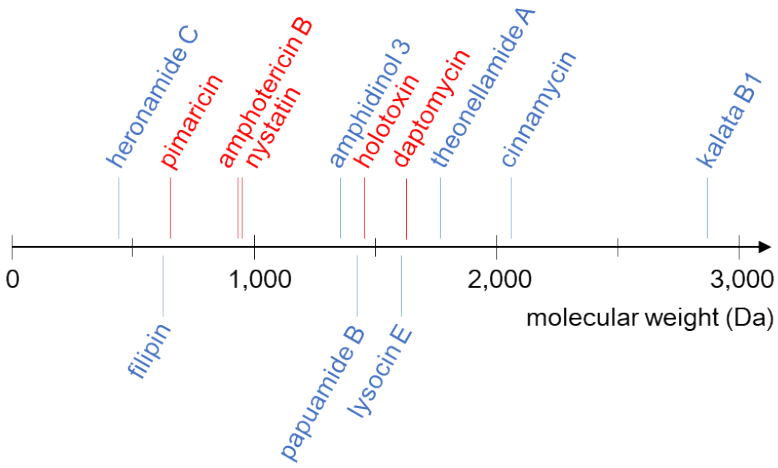


Fig. 5 Molecular weight of the membrane-targeting natural products described in this article. Compounds colored in red are used as clinical agents.

4. Interaction mode between natural products and the lipid membrane

Chemical structures of membrane-targeting natural products are variable in regard to their biosynthetic origin; they include polyketides, terpenes and peptides. In addition, membrane-targeting natural products show variations in their molecular weight (Fig. 5). This could be due to their binding modes as depicted in Fig. 6. Type A: the polar head group of the membrane lipid is recognized from outside the membrane. Type B: the hydrophobic portion of the lipid is recognized through lateral interactions. Type C: compounds reside on/in the membrane owing to their amphiphilic or hydrophobic nature. Compounds categorized in type C usually do not require interactions with specific lipid species for their membrane interaction.

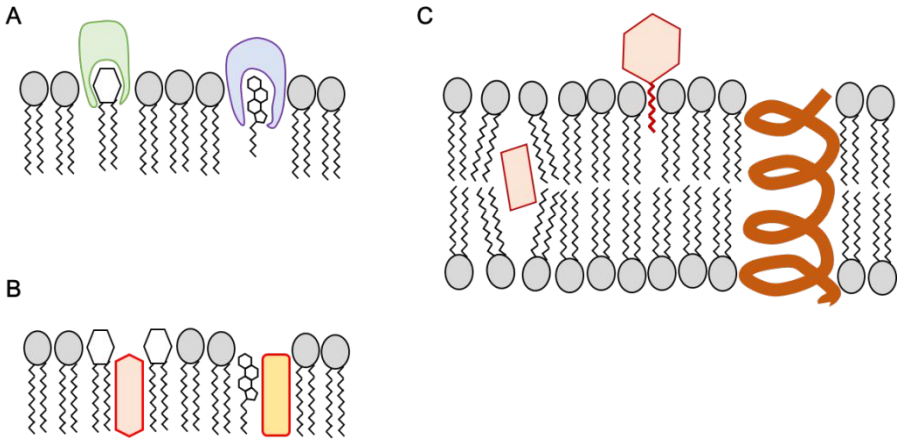


Fig. 6 Molecular modes for lipid recognition by natural products. (A) Type A molecule recognizes lipids at a shallow area of the membrane through vertical interactions. In this case, the head group of phospholipids or the 3-hydroxylated tetracyclic structure of the sterols are recognized by natural products, such as cinnamycin or theonellamide, respectively. (B) Type B molecules bind to lipids through lateral interactions, e.g. heronamide (left) and amphotericin B (right). (C) Type C natural products that bind to the lipid membrane with low lipid specificity are shown. A hydrophobic molecule can be buried in the lipid bilayer (left). Lipopeptides, such as syringomycin E (middle) and helix-forming peptides such as polytheonamide B (right), are included in type C. In all cases, the consequence of the lipid–natural product interaction is not shown, such as lipid extraction, membrane lysis or pore formation.

Molecules categorized in type A have to be large enough to form a cavity in which the head group of the phospholipids or sterols are embedded (Fig. 6). This type includes cinnamycin and duramycin, which recognize phosphatidylethanolamine

(section 5.3.), and theonellamides, which binds to 3β-sterols (section 5.5.). Although the interaction mode at an atomic resolution is not known, papuamide, which targets phosphatidylserine, is likely a member of type A because this molecule does not recognize phosphatidylcholine or phosphatidylethanolamine (section 5.4.). A type A molecule can be larger than the above lantipeptides: kalata B1, a member of cyclotides, has a PE-binding property (Fig. 3, section 5.3).[78-79]

Type B includes polyene macrolide amphotericin B and polyene macrolactam heronamides. Amphotericin B recognizes fungal ergosterol, while heronamides favor saturated hydrocarbon chains of phospholipids. Their binding mode is highly specific, which induces drastic cellular phenotypes. In addition, amphotericin B is used as an antifungal drug. The mechanism of action of type B molecules is described in the following sections in detail (section 5.1 and 5.2). Several saponins are also expected to be type B sterol binders, and their stoichiometry was assessed to be 1:1 for some plant saponins.[11, 80-82] Stichloroside, a sea cucumber-derived saponin was recently shown to target membrane ergosterol by chemical genomic analysis (Fig. 7),[20] while its related metabolite holotoxin, also isolated from a sea cucumber, is used as a main constituent in anti-athlete's foot drugs.[68] The number of natural saponins is huge; e.g. more than 700 triterpene glycosides have been reported from sea cucumbers and more than 1000 from marine organisms.[8-11] Their minor structural differences may affect their molecular and biological activities. We don't include this group of metabolites because mechanisms are not fully unraveled.

Type C compounds show wider variations in their chemical structures compared with the compounds of types A and B. Molecules of any size can be localized in lipid bilayers if they have proper hydrophobic or amphiphilic properties. Small hydrophobic molecules can reside in lipid membranes, for example, local anesthetics.[83] Such properties of natural products have not been investigated thoroughly. Middle-sized molecules include lipopeptides such as syringomycin E (see Section 5.6) and linear, helix-forming peptides like alamethicin.[25] Polytheonamide B, a marine sponge-derived 48-mer peptide, forms a helical structure in lipid membranes, thus working as an ion channel.[84-87] In this article, we do not mention natural products categorized in type C, which usually do not show interactions with specific lipids. We only briefly describe syringomycin E, which shows lipid specificity *in vivo*, but no specificity *in vitro* (section 5.6).

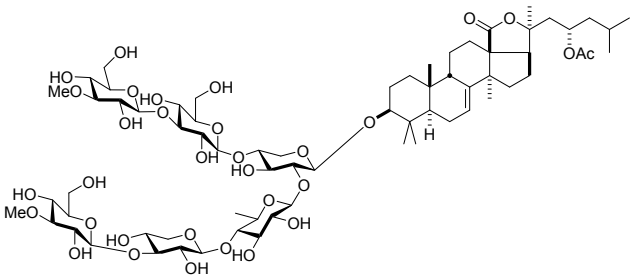


Fig. 7 Chemical structure of a marine-derived saponin, stichloroside C1.

5. Mechanistic insight of membrane-targeting natural products

5.1. Polyene macrolides, targeting sterols.

Polyene macrolide antibiotics are a class of natural products that target fungal cell membranes.[88] Polyene antibiotics have a ring structure in which a conjugated double bond system is located opposite to a number of hydroxy functions. Often a mycosamine group is present in combination with a carboxy moiety, rendering the molecule amphoteric. These polyene antimycotics are typically obtained from some species of *Streptomyces* bacteria. Several members of this class, such as amphotericin B (AmB), nystatin, and pimaricin (natamycin) (Fig. 8), are important antifungal agents and have been clinically used on the basis of their broad spectrum and relatively rare induction of resistance.[89] Although filipin III is also a representative polyene macrolide, it is not used clinically due to its toxicity (Fig. 8). Filipin III, usually used as a mixture of four filipins, is widely used as a probe for sterols in biological membranes because of its fluorescence.[58] The common feature of AmB, nystatin, and pimaricin is the affinity toward ergosterol (Fig. 1) existing in the fungal cell membrane, but their modes of action differ from each other. The larger polyene antibiotics AmB and nystatin are believed to form barrel-stave type transmembrane pores together with ergosterol in fungal cell membranes and collapse vital ion gradients, thereby killing the fungal cells.[90-91] However, although pimaricin also specifically binds to ergosterol, it does not change the permeability of the fungal plasma membrane.[92] Breukink et al. proposed that the depletion of ergosterol by pimaricin inhibits ergosterol-dependent membrane proteins such as glucose and amino-acid transporters, thus eliciting its fungicidal activity.[93] A similar model was recently proposed for AmB by Burke et al.;[94-95] in the so-called sterol sponge model, extramembranous aggregates of AmB physically extract ergosterol from lipid bilayers and thereby kill fungi without them forming pores. In this way, controversy still persists regarding the mechanisms of action of polyene antifungals; however, the general consensus is that the compounds elicit their selective toxicity toward fungi because of their ergosterol-binding properties. Among the above representative polyene macrolides, AmB is the most widely used for the treatment of fungal infections, and its mode of interaction with sterol has therefore been extensively studied. In this section, we introduce the sterol binding mechanism of AmB provided by recent studies.

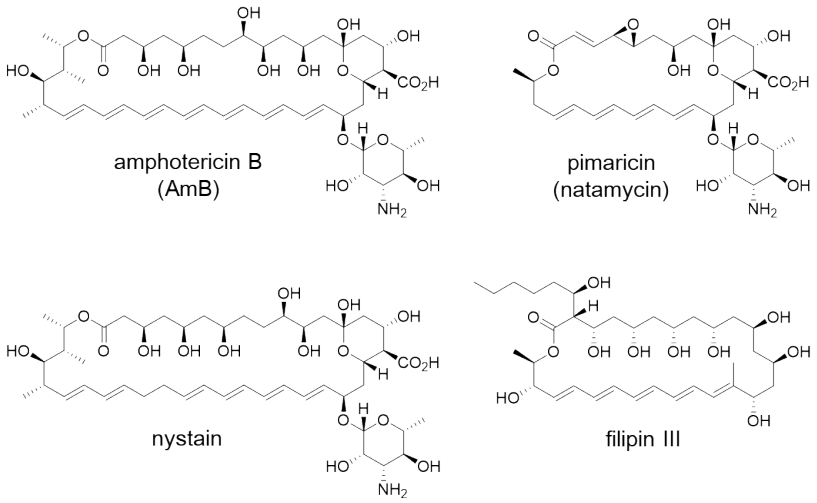


Fig. 8 Chemical structures of representative polyene macrolides, amphotericin B (AmB), nystatin, pimaricin (natamycin) and filipin III.

As mentioned above, although AmB is believed to form transmembrane ion channels with sterols, thus leading to cell damage or cell death, Burke et al proposed a different mechanism in which AmB aggregates extract sterols from cell membranes, like a sponge, and suppresses the function of sterol, leading to cell damage or cell death.[94-95] In any mechanism, AmB's selective toxicity to fungi is attributed to its higher affinity to ergosterol existing in fungal membranes than to cholesterol in mammalian membranes. However, it is still unclear how AmB recognizes the minute structural difference between cholesterol and ergosterol (Fig. 1). To unveil the mechanism underlying its sterol selectivity, three strategies were adopted by Murata and colleagues. First, the solid-state ^{2}H NMR of deuterated cholesterol and ergosterol was measured to observe the change in the sterol mobility depending on the presence and absence of AmB.[96] Second, the structure-activity relationship using synthesized ergosterol analogs was studied.[97-98] Then, intermolecular distances between ^{13}C-enriched ergosterol and ^{19}F-labeled AmB were measured using a solid-state NMR technique, REDOR (rotational echo double resonance).[99-100]

The solid-state ^{2}H NMR signal of deuterated sterol represents its motional property in the membrane. When deuterated sterol undergoes fast axial rotation in membranes, the solid-state ^{2}H NMR spectrum gives a narrow quadrupole splitting. Meanwhile, when sterol's rotation is decelerated or immobilized by interaction with AmB and consequently its rotational time scale is reduced to be comparable to or less than the ^{2}H NMR time scale (μs), the ^{2}H NMR spectrum provides a broadened signal or the largest quadrupole splitting. Based on such spectral features of ^{2}H NMR, it is possible to detect

the interaction of deuterated sterols with AmB. Fig. 9 shows ^{2}H NMR spectra of 3-*d*-cholesterol and ergosterol in the POPC membrane. The spectral feature of 3-*d*-cholesterol was not changed by the presence of AmB, indicating that the fast rotation of cholesterol was less affected by AmB. However, the signals of 3-*d*-ergosterol became broadened, which suggests that the rotational motion of ergosterol in the membrane was hampered by interacting with AmB. Some previous reports proposed that the higher activity of AmB in ergosterol-containing membranes is attributed not to the direct molecular interaction but to the change of membrane physico-chemical properties by the presence of ergosterol;[101] however, this experiment unambiguously demonstrated the direct molecular interaction between AmB and ergosterol in membranes.

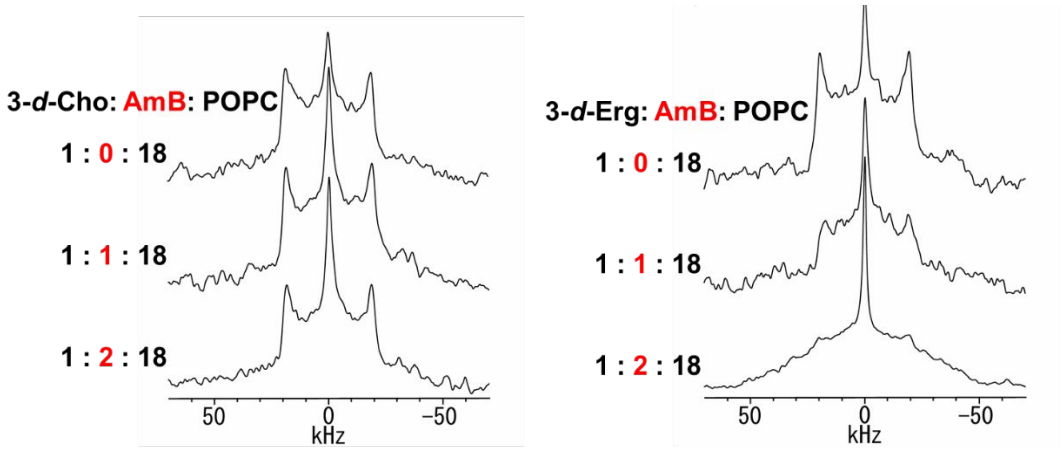


Fig. 9 Solid-state ^{2}H NMR spectra of 3-*d*-cholesterol (left) and 3-*d*-ergosterol (right) in POPC with increasing ratios of AmB. Aadapted with permission from ref. 96. Copyright 2009 American Chemical Society.

The structure-activity relationship (SAR) of sterol was investigated to gain insight into the molecular recognition between AmB and ergosterol.[97-98] The structural difference between ergosterol and cholesterol is in that ergosterol has two additional double bonds in the B ring and the side chain and one additional methyl group on the side chain (Fig. 1). To reveal which portion is responsible for the higher affinity of ergosterol with AmB, ergosterol analogs were prepared and AmB's membrane permeability of K^{+} ions was evaluated in the membranes containing those sterols (Fig. 10). In the K^{+} permeability assay, the liposomes have both pH and K^{+} concentration gradients, and when AmB channels enhance the influx of K^{+} ions, H^{+} efflux concomitantly occurs due to the presence of a proton carrier to maintain the intraliposomal electrical neutrality. The resultant intraliposomal pH rises were detected by a pH dependent fluorescent dye. Fig.

10A shows the channel activity of AmB in the membranes containing ergosterol analogs varying in the alicyclic double bonds.[98] Interestingly, the Δ7 derivative has a comparable activity to ergosterol. Another intriguing result is that the Δ5,7,9 derivative, which has an additional double bond, significantly reduced AmB channel activity. To interpret these results, the conformations of these ergosterol analogues were investigated (Fig. 10A). The conformation of the Δ5,7,9 derivative was found to be twisted, which should hamper close contact with AmB. The conformations of the remaining three analogs appear similar, but the major structural alteration between the low affinity analog (Δ5) and the high affinity sterols (Δ5,7 and Δ7) is in the presence or absence of the axial hydrogen atom at C7 (H7ax). Therefore, the axial hydrogen likely prevent by sterical hindrance a close contact with the AmB macrolide. Taken together, it is suggested that face-to-face VDW interaction between this face of steroid and AmB is important for the molecular interaction.

A similar experiment using ergosterol analogs varying in the side chain was also conducted (Fig. 10B), which revealed that any alteration in the ergosterol side chain significantly reduced AmB channel activity.[97] This suggests that AmB directly recognizes the ergosterol side chain structure. The conformational analysis further showed that the C24-C25 double bond restricts the conformational alteration of the side chain and the C26 methyl group increases the contact area with AmB, both of which increase the van der Waals contact with AmB. The ergosterol derivative having conjugated double bonds in the side chain, which at first we expected to have some activity, exhibited a significantly reduced AmB channel activity. Its side chain conformation was shown to be twisted (Fig. 10B), which would reduce the affinity with AmB.

The SAR study of ergosterol showed that AmB recognizes both steroid rings and the side chain of ergosterol. The study also revealed that AmB binds preferentially to smooth and flat steroid rings (without distortion and axial H-7) as well as a rigid, extended, and methylated side chain. From these findings, it is suggested that ergosterol should interact with AmB in a face-to-face manner to maximize the van der Waals contact as schematically shown in Fig. 10C.

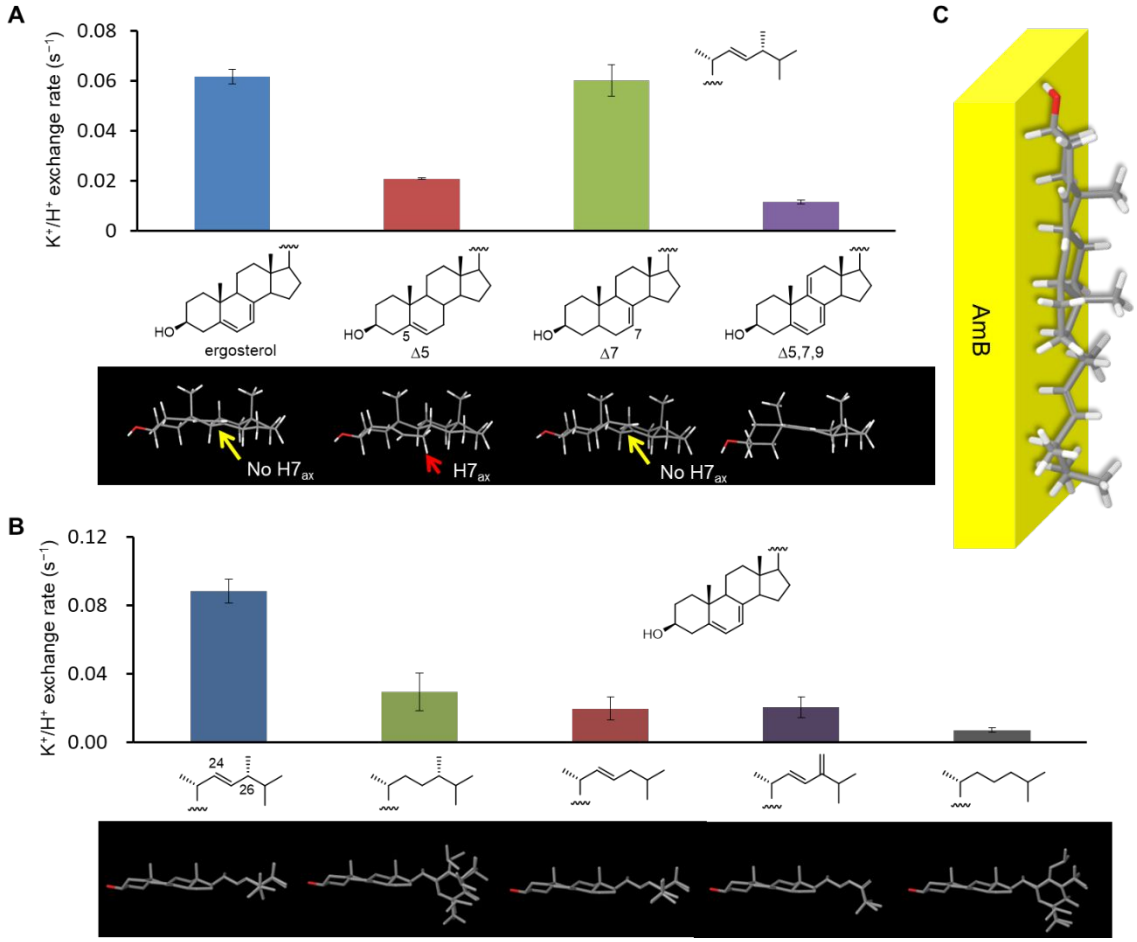


Fig. 10 Structure-activity relationship study of ergosterol analogs varying in the alicyclic double bonds (A) and in the side chain (B). Structures in panel (B) are superposition of conformations within 5 kJ mol–1 calculated by a conformational search algorithm. Panel (C) represents a schematic model of AmB–ergosterol interaction proposed on the basis of the SAR studies. Adapted with permission from refs. 97 and 98. Copyright 2014 and 2015 American Chemical Society.

In the third approach, intermolecular distance information was estimated by the Rotational Echo DOuble Resonance (REDOR) method.[99-100] The REDOR method is a versatile solid-state NMR technique to measure heteronuclear dipole interactions, from which internuclear distances can be evaluated.[102] In the case of ^{13}C-^{19}F REDOR, it is possible to measure distances between ^{13}C and ^{19}F atoms up to 1 nm. 14-F-AmB, a fluorinated derivative of AmB,[103] and ^{13}C-ergosterol, in which ^{13}C-labels were biosynthetically incorporated in a skipped manner, were prepared and the ^{13}C-^{19}F REDOR was measured in POPC bilayers (Fig. 11A).[99] The REDOR spectrum showed that the14-F atom is close not only to the C19 angular methyl group but also to the C26,27

terminal dimethyl group, suggesting that the AmB–ergosterol interaction occurs not only in a parallel manner but also in an anti-parallel orientation. This result was totally unexpected as all the previous AmB-ergosterol interaction models only assumed parallel alignments.[90] To confirm the presence of the anti-parallel interaction as well as to extract the intermolecular distances more accurately and precisely, 26,27-$^{13}C_2$-erogsterol, 4-^{13}C-ergosterol, and 37-F-AmB were synthesized and ^{13}C-^{19}F REDOR measurements were conducted for the four combinations; 37-F-AmB/26,27-$^{13}C_2$-ergosterol, 14-F-AmB/26,27-$^{13}C_2$-ergosterol, 14-F-AmB/4-^{13}C-ergosterol, and 37-F-AmB/4-^{13}C-ergosterol.[100] These REDOR analyses revealed that parallel and anti-parallel orientations coexist at the ratio of 7:3, and the precise intermolecular ^{13}C-^{19}F distances are shown in Fig. 11B. Based on the distance information, a geometry search for AmB–ergosterol complexes was examined, which proposed the most likely geometries for the parallel and anti-parallel orientations (Fig. 12), by taking account of the face-to-face interaction between AmB and ergosterol that was suggested from the above SAR study. Thus the answer to why the AmB–cholesterol interaction is weaker than that of AmB–ergosterol is that the presence of H-7ax as well as the flexible and non-methylated side chain weakens the van der Waals contact of cholesterol with AmB.

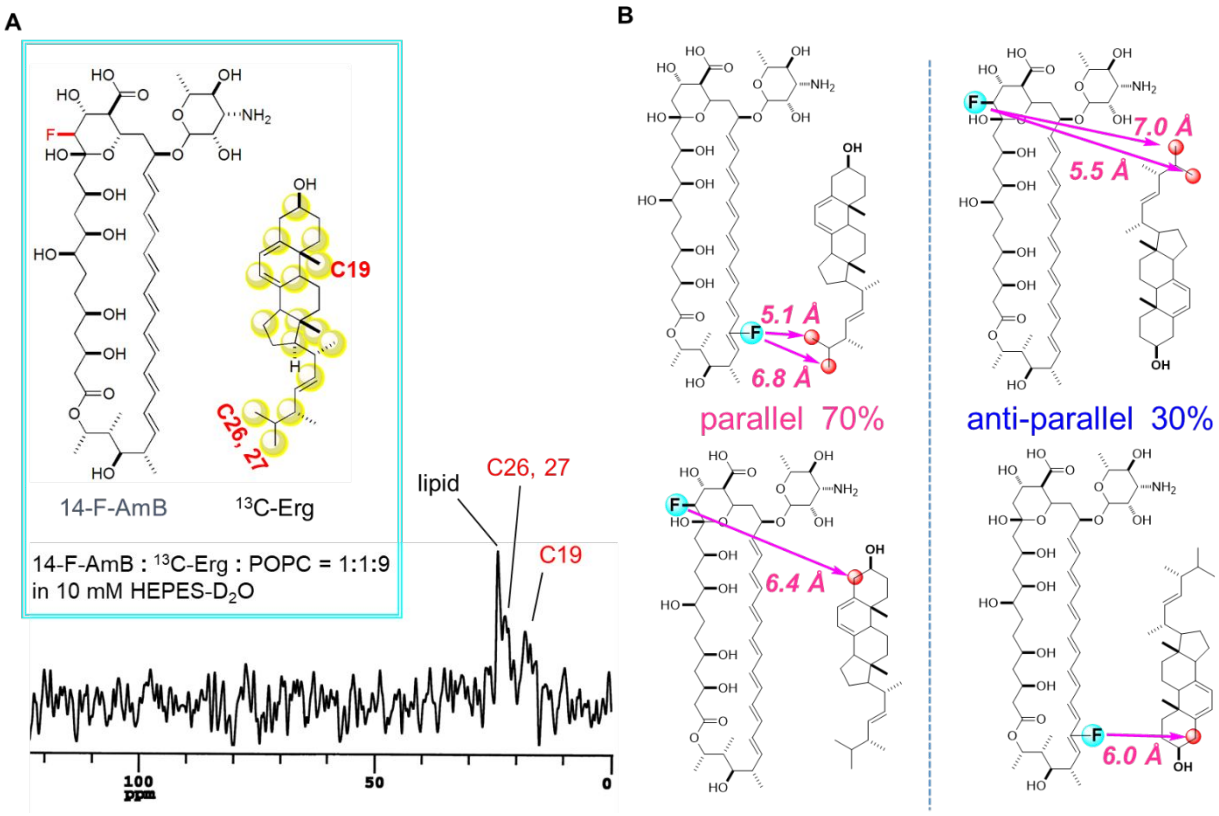


Fig. 11 REDOR measurements of fluorinated AmB and ^{13}C-labeled ergosterol. (A) REDOR between 14-F-AmB and biosynthetically ^{13}C-labeled ergosterol. Carbons highlighted in yellow indicate ^{13}C-labeled positions. The bottom figure represents the REDOR difference spectrum, which suggests that C19, 26, and 27 carbons are close to the 14-F atom. (B) Combinations between fluorinated AmB and chemically synthesized ^{13}C ergosterol for REDOR measurements, as well as the ratio between parallel and anti-parallel orientations and internuclear distances. Adapted with permission from ref. 99. Copyright 2012 American Chemical Society.

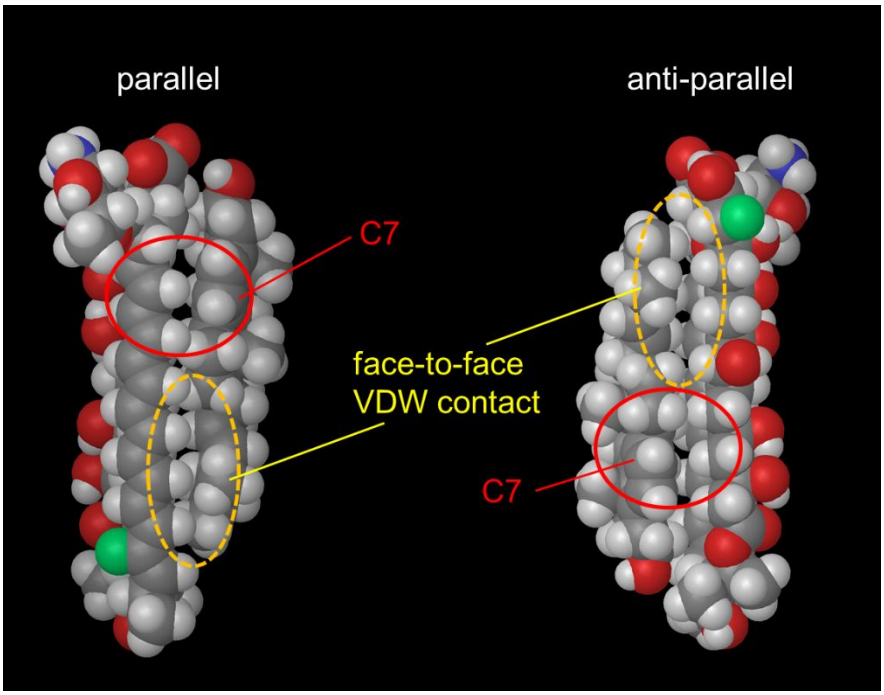


Fig. 12 Models for parallel and anti-parallel alignments of AmB-ergosterol interaction.

5.2. Heronamides, targeting phospholipids with saturated hydrocarbon chains.

Another class of cyclic polyketide compounds that target the cell membrane are polyene macrolactams, represented by heronamides (Figs. 13, 14). More than ten macrolactams with varying sizes have been reported; cyclamenol has a 20-membered ring, micromonosporin A has a 24-membered ring, and salinilactam A has a 26-membered ring.[104-110] The macrolactam ring is decorated by hydroxyl groups, methyl groups, and/or hydrocarbon chains. Glycosylation of the hydroxyl group is not rare (Fig. 13B).[111-114] Polyene macrolactams often show intramolecular cyclization yielding polycyclic structures.[114-116] In Figs. 13 and 14, we show only compounds with a monocyclic structure. The compounds can be divided into three groups based on the starter unit of their biosynthesis. First, cyclamenol, the aglycon of vicenistatin, and sceliphrolactam have methyl substitutes at positions 18, 18 and 24, respectively; this type of compound uses 3-methylasparatate as a starter unit.[114-115, 117-118] Second, micromonosporin A and the four 26-membered macrolactam compounds illustrated have methyl substitutes at position 23 and 25, respectively; they seem to be synthesized using 3-aminobutyrate as a starter unit.[106, 110, 119] Finally, aureoverticillactam and 20-membered macrolactams such as heronamides (Figs. 13, 14) have a hydrocarbon chain adjacent to the amide group; this group uses a β-aminoacyl starter unit.[120-122]

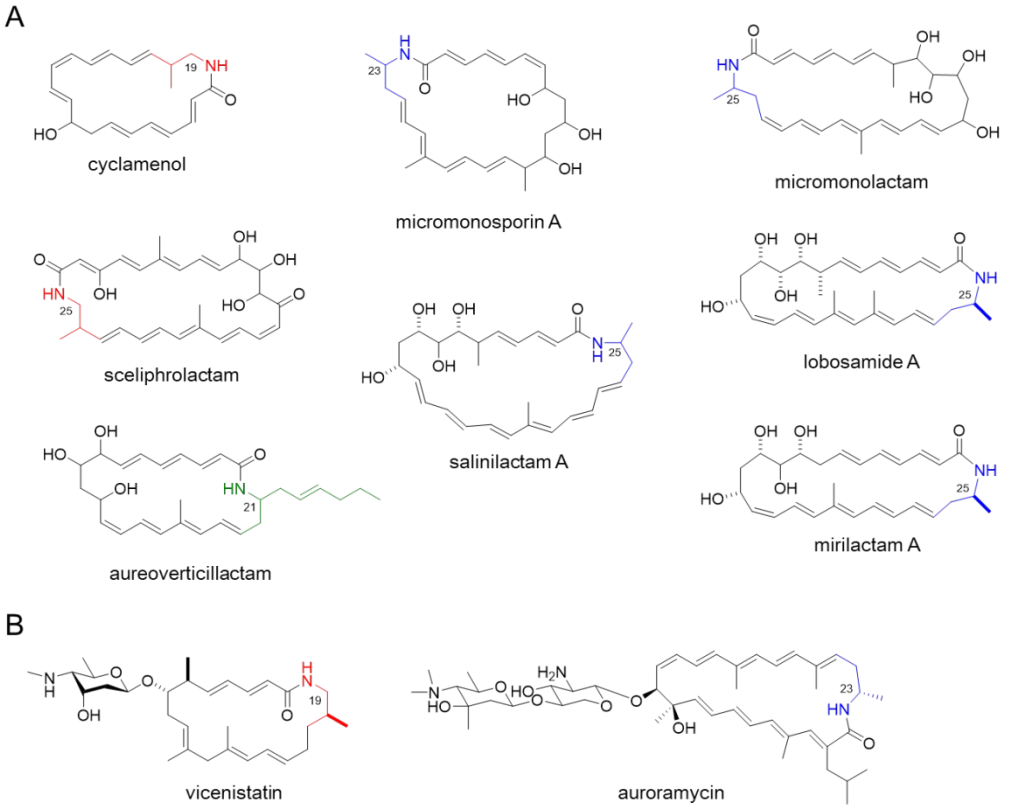


Fig. 13 Chemical structures of polyene macrolactams. Macrolactams without a sugar appendage (A) and glycosylated macrolactams that appear in this article (B) are shown. Starter units in the biosynthesis are colored: 3-methylasparatate (red), 3-aminobutyrate (blue), ß-aminoacyl (green).

The heronamide-class of macrolactams include BE-14106/GT32-A[123] and the 8-deoxy congener GT32-B[124], ML409[121], heronamide C[125] and F[126], and 8-deoxyheronamide C,[72] all of which were reported from *Streptomyces* species (Fig. 14). These compounds are relatively unstable and intramolecular cyclization easily furnishes tri- or tetra-cyclic compounds, such as heronamides A and B (Fig. 14).[125-131] In the ^{1}H NMR spectra of these compounds, olefinic protons give heavily overlapping signals, hampering correct assignment of the stereochemistry of the compounds.[125] In contrast, polycyclic compounds produced from the monocyclic compounds show relatively dispersed olefinic protons, for example, heronamide A from heronamide C.[127] Taking advantage of this conversion, the relative and absolute stereochemistry of heronamide C was determined, which was confirmed by total synthesis.[72, 128]

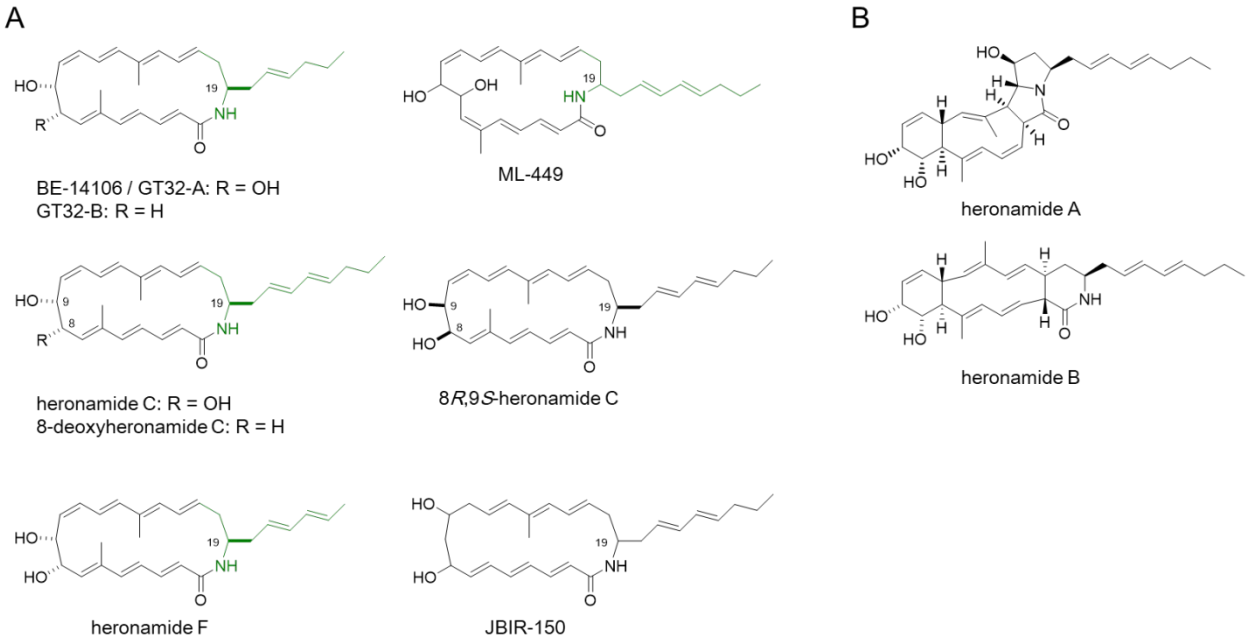


Fig. 14 Chemical structures of BE-14106, heronamides and related metabolites. (A) Metabolites with a mono cyclic structure are shown. Starter units in the biosynthesis are green. (B) Chemical structures of heronamides A and B.

Various biological activities have been reported for polyene macrolactams: antibacterial[123-124], antifungal[72, 120, 123], antitumor activities[132],[111], inhibition of leukocyte adhesion,[133] antiproliferative activity against mammalian cells[120, 124, 134], inhibitory activity against mixed lymphocyte reaction[124], and reversible vacuolation in HeLa cells[125]. Among polyene macrolactams, heronamide C and its 8-deoxy congener show potent antifungal activities and tight membrane-binding activities[72].

The clue for the cellular target of heronamide C and the 8-deoxy congener was obtained by characteristic chemical genetic interactions: fission yeast cells lacking ergosterol biosynthetic genes were tolerant to 8-deoxyheronamide C, which suggested that these compounds target the cell membrane. Cells lacking ergosterol biosynthetic genes are tolerant to membrane-binding compounds, such as polyene macrolides and syringomycin E.[135-136]

Physical interactions between lipid membranes and heronamides were analyzed by surface plasmon resonance (SPR) experiments.[72] Liposomes were immobilized on the sensor chip, and compounds were eluted as analytes. The sensorgrams revealed that heronamides bind to liposomes consisting of phospholipids with saturated hydrocarbon chains such as 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and sphingomyelin (SM) (Fig. 15). This binding was irreversible as no release

of analyte from the sensor chip was observed after stopping the elution of heronamides. In contrast, heronamides showed only weak binding to liposomes consisting of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) or 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (Fig. 15). The membrane binding activity of heronamide C and 8-deoxyheronamide C was not affected by the presence of 20 mol% of ergosterol in the liposome. These experiments were conducted at 30°C. At this temperature DMPC membranes display a liquid disordered phase whereas DMPC membranes containing cholesterol have a mixture of liquid disordered and ordered phases. SM membranes are in a gel phase. This indicates that the membrane fluidity is unlikely to affect the affinity of heronamides. Rather, heronamides seem to favor lipid molecules possessing saturated hydrocarbon chains. The molecular size of heronamides suggests that they can act as pseudo-sterols (Fig. 15). Cholesterol, for example, has a preference for interaction with lipids that have fully saturated hydrocarbon chains when compared with lipids that have unsaturated chains.[39, 137-139]

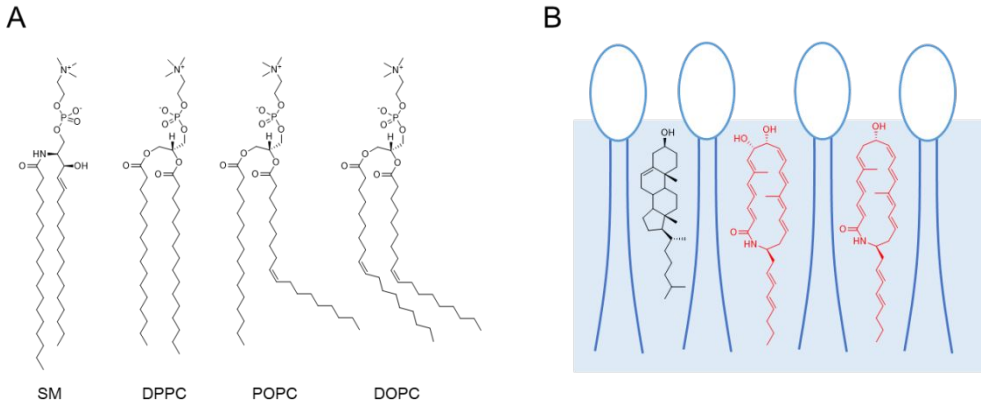


Fig. 15 Plausible model of the interactions between lipids and heronamides. (A) Chemical structures of phospholipids are shown. The left two (SM and DPPC) showed a high affinity to heronamides, and the right two (POPC and DOPC) showed weak affinity. (B) A schematic for the interactions between phospholipids, cholesterol and heronamides. Heronamides likely are buried in the lipid bilayer, in a similar fashion with cholesterol.

Structure-activity relationships found in heronamide congeners seem to support the pseudo-cholesterol model of heronamides (Fig. 16); the hydrophobic ring and tail are inserted into the lipid membranes, whereas hydroxyl groups are located in the shallow area of the membranes. The presence of hydroxyl groups might stabilize the lateral interaction between heronamides and phospholipids, probably by hydrogen bonding with lipid head groups, which can explain the difference in the potency of heronamide C and

8-deoxyheronamide C. Heronamide C showed about 10 times higher membrane binding activity than 8-deoxyheronamide C. In addition, heronamide C, whose anti-yeast activity was comparable to that of amphotericin B, showed 20–40 times more potent anti-yeast activity than 8-deoxyheronamide C (Fig. 16). When hydroxyl groups in heronamide C were acetylated, the compound lost membrane affinity and anti-yeast activity. The importance of the intact macrolactam ring was shown by the significant loss of biological activities of heronamide A and heronamide B.[72] In addition, 8*R*,9*S*-heronamide C, a synthetic congener, was 80 times less active than heronamide C.[128, 140] This synthetic analog showed opposite Cotton effects in the CD spectrum comparable with natural heronamide C, which suggests that the conformation of the macrolactam ring is important for these molecules to be embedded in the membrane. Otherwise, the direction of the hydroxyl groups in the 8*R*,9*S*-heronamide C was not in a proper position for exhibiting potent membrane affinity. The structure of the tail is correlated with antifungal activity. BE-14107, which has a shorter hydrocarbon tail by one acetate unit, showed four times less potent activity. The strength of the hydrophobic interaction between the tail of heronamides and the saturated hydrocarbon chain of the phospholipid could be easily regulated by the structure/length of the tail.

compound	MIC (μM)
heronamide C	0.13-0.28
synthetic heronamide C	0.13
BE-14106	0.50
8-deoxyheronamide C	5.8
8S,*9R*-heronamide C	10
heronamide B	50
heronamide A	N.A. at 40 μM
heronamide C diacetate	N.A. at 40 μM
amphotericin B	0.27

Fig. 16 Structure-activity relationships of heronamides. Growth inhibitory activity of heronamides against wild-type fission yeast cells is compared.[72, 128, 141] N.A.: not active.

Capon and colleagues reported that heronamide C induces vacuolation of HeLa cells without toxicity.[125] This drastic morphological change was reversible; after washing out the compound, cells showed normal shape. Although the molecular basis for this unique phenomena has not been reported, SM is the candidate target molecule because heronamide C showed tight binding to liposomes consisting of SM or SM and 20 mol% cholesterol.[72]

Heronamides induced drastic morphological changes also in the fission yeast:

accumulations of cell wall material at both cell tips and septa were observed (Fig. 17). This material might be 1,3-β-glucan because genetic perturbation of Bgs1 and Rho1 counteracted this phenotype. Bgs1 is one of four 1,3-β-glucan synthases in this organism,[142] while Rho1 is an essential protein for activating 1,3-β-glucan synthase.[143] A similar phenotype was reported by perturbation of sphingolipid metabolism. *css1* temperature-sensitive mutant cells display similar morphological changes when placed under restriction temperature; the *css1* gene encodes sphingolipid-phospholipase C.[144] Glycerophospholipids of fission yeast contain primarily 18:1 fatty acyls[145]. In contrast, the predominant type of sphingolipid was reported to be t18:0/26:0, consisting of phytosphingosine and a long saturated fatty acid. Curiously, a similar phenomenon was observed when cells were treated with theonellamide, whose cellular target is ergosterol (see section 5.5).[21] All these data suggest that the cell wall biosynthesis machinery is regulated by a so-called lipid raft, a membrane micro-domain consisting of sphingolipids, sterols, and/or proteins (Fig. 17).[46] In fact, Bgs1 is a membrane protein containing multiple transmembrane helices and was shown to be insoluble in non-ionic detergents[146], one characteristic of lipid-raft proteins. Detailed cellular and biochemical analyses underlying the abnormal cell wall biosynthesis induced by two classes of natural products would unveil the novel function of membrane lipids.

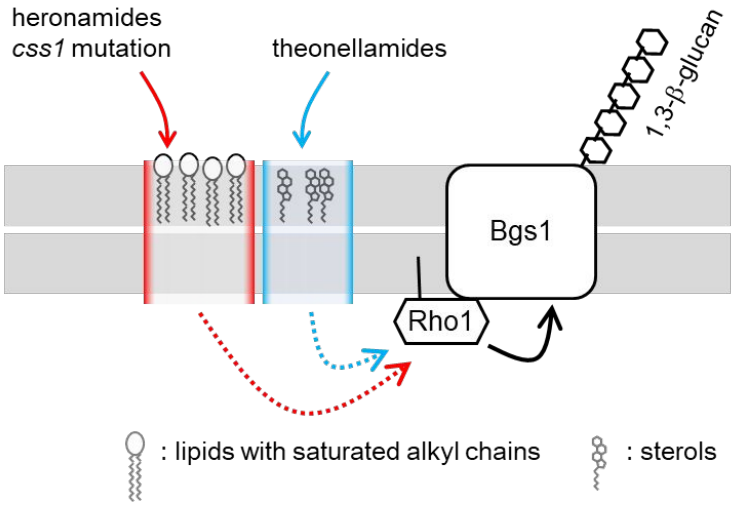


Fig. 17 Model for abnormal cell wall accumulation by heronamides and theonellamides in fission yeast. Heronamides and theonellamides bind to membrane lipids, then induce cells to synthesize a thick cell wall. This phenotype is attenuated in *rho1* or *bgs1* mutant cells. Similar phenomena were reported by a mutant of *css1* gene, encoding sphingolipid phospholipase C.

So far cellular targets responsible for the biological activities of polyene macrolactams other than heronamides are missing. Recently, vicenistatin and auroramycin, both of which have a sugar decoration, were reported to modulate membrane-related events in mammalian and yeast cells.[147-148] As they can have non-proteinous cellular targets like the heronamides do, non-targeted methods for target identification are preferred for the studying biological activity of polyene macrolactams.

5.3. Cinnamycin, duramycin and cyclotides, targeting PE.

Cinnamycin (Ro 09-0198) and duramycin are tetracyclic peptides consisting of 19 amino acids (Fig. 18), which are produced by *Streptomyces* species and *Streptoverticillium cinnamoneus*, respectively.[149-150] They are members of the lantibiotics family compounds, which are ribosomally synthesized peptides that are post-translationally modified to possess thioether cross-linked amino acids lanthionine and methyllanthionine. Cinnamycin and duramycin have one more unusual bridge, lysinoalanine. They differ from each other by a single amino acid: arginine for cinnamycin and lysine for duramycin. These two lantibiotic peptides specifically bind to phosphatidylethanolamine (PE) and the best characterized compounds targeting PE.[151]

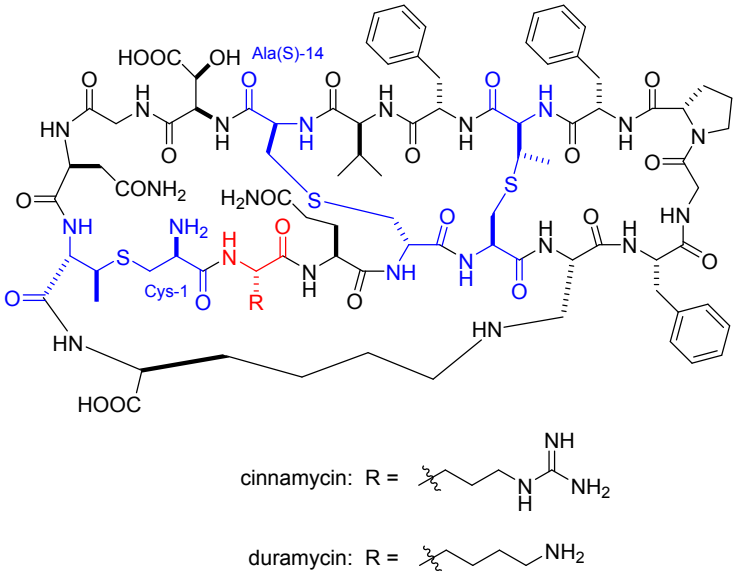


Fig. 18 Chemical structures of cinnamycin and duramycin. One lanthionine and two methyllanthionine residues are shown in blue. The structural difference between cinnamycin and duramycin is one amino acid residue (red). Free amino groups (Cys-1 for both compounds, and Lys-2 for duramycin) can be chemically modified for installing reporter groups without losing PE-binding activity.

Cinnamycin and duramycin are globular molecules. Both compounds specifically bind to ethanolamine phospholipids such as diacyl PE, plasmalogen PE and lyso-PE (Fig. 19A).[152] The binding constant K_a of cinnamycin and duramycin towards PE in POPC:POPE liposomes is 6×10^7 M^{-1} and 2.1×10^8 M^{-1}, respectively.[152-153] These peptides form a 1:1 complex with PE.[153-154] The solution structure of cinnamycin complexed with lyso-PE was deduced in DMSO by ^1H NMR (Fig. 19B).[154-155] The peptide had a hydrophobic pocket surrounded by residues Phe-7 through Ala(S)-14 to bind to the head group of the ligand. This pocket is supported by four intramolecular bridges: one

lanthionine residue, two methyllanthionine residues and one lysinoalanine residue. Intermolecular contacts were observed between ethanolamine CH_2N methylene and Gly-8 methylene, glycerol C3 methylene and Val-13 methyl, glycerol C3 methylene and Pro-9 C_δ methylene, and glycerol C2 methine and Val-13 methyl. In addition to these hydrophobic interactions, the complex is also stabilized by an ionic interaction between the carboxylate group of HO-Asp-15 and the ammonium group of lysoPE.

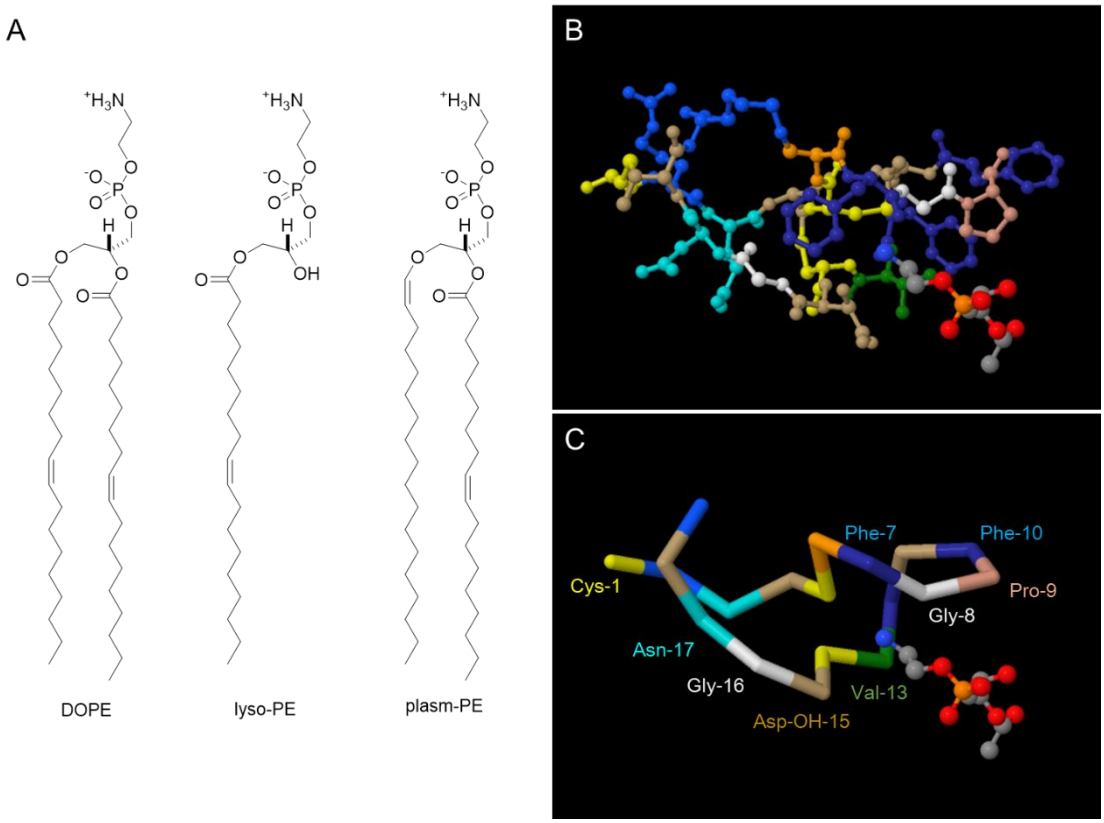


Fig. 19 Structures of ethanolamine phospholipids and cinnamycin complexed with lysophosphatidylethanolamine. (A) Chemical structures of phosphoethanolamine-containing lipids. DOPE: 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; lyso-PE: 1-oleoyl-2-hydroxy-*sn*-glycero-3-phosphoethanolamine; plasm-PE: 1-*O*-1'-(*Z*)-octadecenyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine. (B, C) Structures of cinnamycin complexed with lysophosphatidylethanolamine, which was deduced by ^{1}H NMR analysis (PDB: 2DDE). Structures were illustrated by Jmol, an open source Java viewer for chemical structures in 3D: http://www.jmol.org.

Cinnamycin and duramycin recognize PE *in vitro*. However, PE preferentially distributes in the cytoplasmic leaflet of the plasma membrane. For example, 99.8% or

97.7% of PE was located in the inner leaflet of the plasma membrane of human erythrocytes and nucleated cells such as skin fibroblasts, respectively.[156] This contradiction was elegantly elucidated by Kobayashi and co-workers.[152, 157] They showed that cinnamycin and duramycin induced the transbilayer movement of phospholipids such as PC and PE, in cell and model membranes. The flip-flop was dependent upon the amount of PE in the liposome membrane, with as little as 1% PE being effective. At the same time, the shape of the liposomes were drastically altered by inducing membrane tabulation.[152, 157] On the other hand, cinnamycin and duramycin favored small vesicles with high membrane curvatures.[152] Taken together, these peptides may catch a small amount of PE on the outer leaflet of the plasma membrane, then the lipids are turned out to the outer leaflet (Fig. 20). During binding of the peptides to the surface of the membrane, the membrane's shape transforms a high curvature, which these peptides favor.

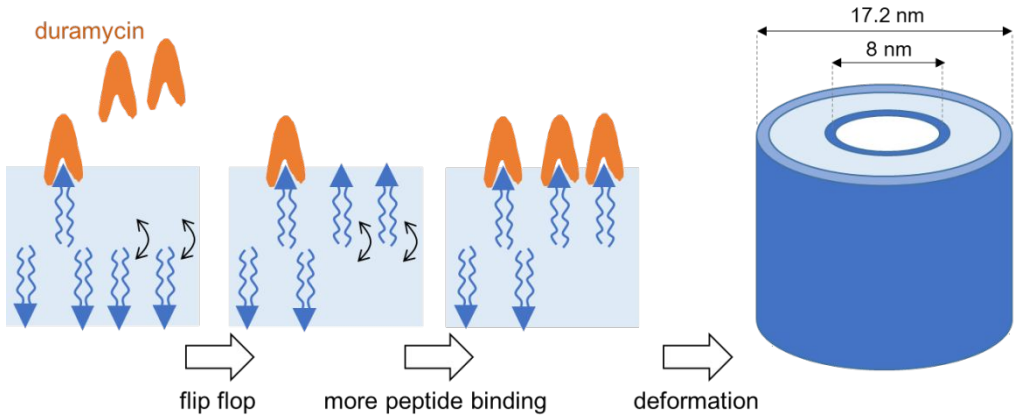


Fig. 20 Schematic for the binding of cinnamycin and duramycin to lipid membranes. PE is recognized by the lantibiotics, followed by a flip-flop of phospholipids (black arrows). In the eukaryotic cell membrane, most of the PE is localized in the inner leaflet. The flip-flop moves the PE from the inner to outer leaflet, thus the binding of the peptides to the membrane continues. Finally, liposomes are deformed to tubular structures that favor the PE on the curvature membrane, which has been increased by the lantibiotics.

Biotinylated cinnamycin has been used to visualize PE in cells and model membranes.[55] Their characteristic phenotype, i.e., the arrest of cytokinesis, revealed the importance of the regulation of cellular localization, while analysis of mutant cells resistant to these lantibiotics unveiled new lipid transport systems.[158-160] These tool molecules can also be used to detect PE *in vivo*. The technetium-99m-labeled duramycin was used to detect the appearance of PE in the infarct after acute myocardial

infarction.[161] *In vitro* and *in vivo* imaging using biotinylated and near-infrared fluorophore-tagged duramycin showed that, PE is exposed on the tumor vascular endothelium.[74] The outer leaflet PE could be used as a biomarker for the tumor vasculature. Based on the established molecular mechanism of PE binding by cinnamycin and duramycin, more applications could be developed.

Cyclotides are found in plants from the Violaceae, Rubiaceae, and Cucurbitaceae families.[162-163] They have six Cys residues forming three disulfide bonds in their ~30 amino acid sequence, while the N- and C-terminus amino acids are conjugated to form another ring (Fig. 21). Cyclotides belonging to subfamilies, termed the brancelet and Möbium subfamilies, generally show affinity to PE.[164] Their rigid structure with a cyclic cystine knot topology, coupled with conserved Glu and Arg/Lys residues, enable their selective binding to PE.[79] Cyclotides exhibit a variety of biological activities including uterotonic, anti-HIV, antitumor, antimicrobial and insecticidal activities, which are thought to depend on their PE-binding activity.[79] The molecular consequence of the binding of cyclotides to PE in the plasma membrane remains to be clarified; however, it has been reported that cinnamycin exhibits somewhat similar activities, such as the transbilayer movement of lipids that was induced by cinnamycin.[79] Other reviews are covering the structural and biological diversity of cyclotides, and their membrane binding abilities.[151, 163-164]

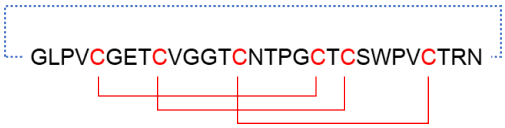


Fig. 21 Structure of kalata B1, a member of the cyclotide family compounds. Cysteine residues and disulfide bonds are shown in red. The blue dotted line shows the amide bond between *N*- and *C*-terminus.

5.4. Papuamides, targeting phosphatidylserine.

Papuamide A and B were isolated from Papua New Guinea collections of the sponges *Theonella mirabilis* and *Theonella swinhoei*.[165] These molecules have a cyclic depsipeptide ring consisting of seven amino acids, to which a linear tetrapeptide is conjugated through an amide bond (Fig. 22). Several amino acids are nonproteinogenic. The *N*-terminus of the tetrapeptide chain was capped by a polyketide chain, 2,3-dihydroxy-2,6,8-trimethyldeca-(4*Z*,6*E*)-dienoic acid. Stereochemical assignments of the amino acids were made by chemical degradation and derivatization of the resulting products, which was followed by chromatographic comparisons of the derivatives with synthetically prepared or commercially available standards. [165-167] Finally, total synthesis by Ma and colleagues established the stereochemistry of these cyclodepsipeptides.[168]

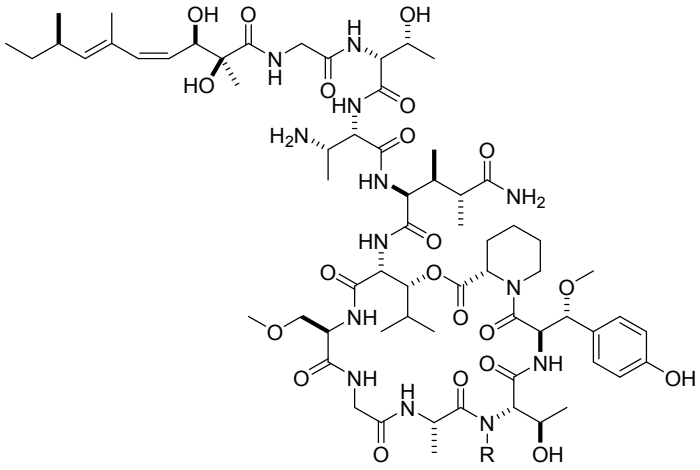


Fig. 22 Chemical structures of papuamide A and B.

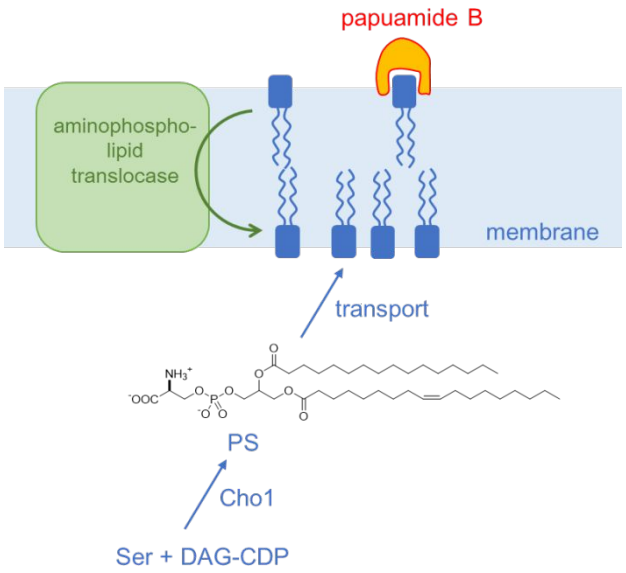


Fig. 23. Schematic of the cellular localization of PS. Papuamide B binds to PS on the cell surface. In genetic screening, a null mutant of the *cho1* gene encoding PS synthase showed tolerance to papuamide B, while cells lacking aminophospholipid translocase conferred hypersensitivity to the peptide because much more of PE is present on the cell surface. DAG-CDP: 1,2-diacyl-*sn*-glycero-3-cytidine-5'-diphosphate.

Papuamides exhibit strong inhibitory effects on the infection of human T-lymphoblastoid cells by HIV-1 RF *in vitro*, with an EC_{50} value of about 3.6 ng/mL. The mechanism of this anti-HIV activity was shown to be through inhibition of virus entry by directly interacting with the virus.[169] Both compounds showed potent cytotoxicity against human cancer cells with IC_{50} values of less than 74 ng/mL. A screening panel of human cancer cell lines revealed a relative sensitivity of multi-drug-resistant cell lines and a relative resistance of leukemia cell lines to papuamide A. The target molecule in the anti-HIV activity and cytotoxicity remains to be determined.

Papuamide A and B show growth inhibitory effects against yeast *Saccharomyces cerevisiae* and *Candida albicans*.[22, 170] The target molecule of papuamides in yeast was revealed to be phosphatidylserine (PS) by employing a budding yeast chemical genomic analysis coupled with a traditional genetic strategy.[22] Parsons *et al.* collected chemical genetic interactions by cross testing 82 compounds and crude natural product extracts with ~4800 haploid deletion mutants [22]. This approach enabled efficient identification of genes whose products buffer the cells from defects in the target pathway of the compound. Papuamide B exhibited more than 300 chemical genetic interactions, which were enriched for genes with certain gene ontology annotations, [171]

including vesicle-mediated transport, cell wall organization and biogenesis, and protein modification. This suggested that this compound may affect intracellular transport or perturb some targets on the cell surface. In addition, the papuamide B chemical-genetic screen was compared to a set of 132 genome-wide genetic interaction screens[172] by hierarchical clustering, which revealed that papuamide B treatment and cell-surface mutants have similar cellular defects.

The direct clue for the cellular target of papuamides in yeast was obtained through the analysis of drug-resistant mutants. Papuamide B-resistant mutants were obtained by spotting wild-type cells on rich medium containing high concentrations of papuamide B. Genetic analysis confirmed that the resistance was associated with a single complementation group, while a gene associated with resistance was cloned by using a plasmid-based genomic library. The gene that was cloned was *CHO1*, and the drug-resistant strain was a *cho1* null mutant. The *CHO1* gene is a nonessential gene, encoding for phosphatidylserine synthase and the null mutant lacks the ability to synthesize PS (Fig. 23).[173-175] Because the chemical genomic analysis suggested that papuamide B targets cell surface molecules, the compound was expected to recognize PS directly.

In the eukaryotic plasma membrane, PS and PE are mainly located in the inner leaflet.[156] This asymmetry is established by aminophospholipid translocases that flip PS and PE from the outer leaflet to the inner leaflet. Yeast mutant cells lacking aminophospholipid translocases, such as Drs2, Dnf1, and Dnf2, expose more PS on the outer leaflet of the cell membrane than wild-type cells (Fig. 23).[176-177] These mutants became super-sensitive to papuamide B.[22] In a marker release experiment using liposomes, papuamide B was about 100-fold more potent against PC liposomes with 10% PS than PC liposomes with 10% PE or pure PC liposomes. These lines of evidence support a mechanism of action for papuamide B, in which it compromises the yeast cell membrane integrity through a direct interaction with PS.

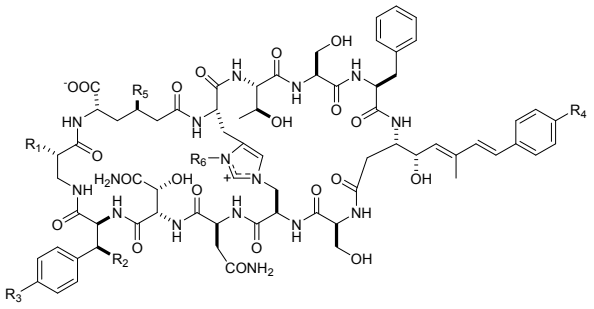
After it was clarified that PS is recognized by papuamide B, this natural product became a unique research tool: the amount of PS in the outer leaflet of the plasma membrane of living cells can be assessed by testing cellular sensitivity to papuamides. This strategy allowed biologists to investigate the cellular function of aminophospholipid translocases[178-179] and to explore new factors involved in the regulation of phospholipid asymmetry.[180-181] In another study, papuamide A was used in a high-throughput phenotypic drug screening that targeted fungal phosphatidylserine (PS) synthase.[170] Compounds that antagonize the growth inhibitory activity of papuamide A against fungi are expected to be drug candidates because PS synthase is essential for virulence in

Candida albicans.[182]

Papuamide B is a rare molecule that recognizes a membrane glycerophospholipid with high specificity. It is noted that there is no information regarding the recognition mechanism of PS by pauamide B. A structure–activity relationship study using synthetic analogs and natural congeners, together with structural analysis of the lipid-compound complex by NMR and X-ray crystallography would help to elucidate the molecular recognition at an atomic level and help unravel the pharmacophore. In addition, the target molecule in the anti-HIV activity is also of interest as it was reported that the activity of papuamides was not due to its PS recognition abilities.[169]

5.5. Theonellamide, targeting 3β-sterols.

Theonellamides (TNMs) are bicyclic peptides isolated from marine sponges of *Theonella swinhoei* (Fig. 24).[183-186] Theonegramide, theopalauamide and isotheopalauamide are related compounds with minor modifications at specific amino acid side chains, which were also isolated from marine sponges of *Theonella swinhoei*.[187-188] These compounds have a characteristic bicyclic structure bridged by a histidinoalanine residue. A subfamily of compounds contains a sugar group on the imidazole ring at the center of the molecule. The bacterial origin of theopalauamide was first inferred by cell fractionation analysis.[189-190] Recently, metagenomics analysis unveiled that the uncultivated Entotheonella sponge symbionts produce theonellamides.[191] The reported biological activities of these compounds, such as their potent antifungal activity, and moderate cytotoxicity, were not dependent on the presence of the sugar group,[183-188, 192-194] suggesting that the characteristic bicyclic peptide framework is responsible for their specific biological actions.



theonellamide A R_1 = OH; R_2 = Me; R_3 = Br; R_4 = H; R_5 = OH; R_6 = β-$_D$-Gal
theonellamide B R_1 = OH; R_2 = Me; R_3 = Br; R_4 = Br; R_5 = OH; R_6 = H
theonellamide C R_1 = H; R_2 = H; R_3 = H; R_4 = Br; R_5 = OH; R_6 = H
theonellamide D R_1 = H; R_2 = H; R_3 = Br; R_4 = Br; R_5 = OH; R_6 = β-$_L$-Ara
theonellamide E R_1 = H; R_2 = H; R_3 = Br; R_4 = Br; R_5 = OH; R_6 = β-$_D$-Gal
theonellamide F R_1 = H; R_2 = H; R_3 = Br; R_4 = Br; R_5 = OH; R_6 = H
theonellamide G R_1 = OH; R_2 = H; R_3 = Br; R_4 = H; R_5 = H; R_6 = β-$_D$-Gal
theonellamide I R_1 = OH; R_2 = H; R_3 = Br; R_4 = H; R_5 = H; R_6 = β-$_L$-Ara
theonegramide R_1 = OH; R_2 = Me; R_3 = Br; R_4 = H; R_5 = H; R_6 = α-$_D$-Ara
theopalauamide R_1 = OH; R_2 = Me; R_3 = Br; R_4 = H; R_5 = H; R_6 = β-$_D$-Gal

Fig. 24 Chemical structures of marine-derived theonellamides.

The cellular target of TNMs responsible for antifungal activity was revealed to be ergosterol by yeast chemical genomic analyses in 2009 and 2010. Boone and colleagues employed a budding yeast system,[20] while Yoshida and colleagues developed a fission yeast system.[21] In the budding yeast omics study, a molecular barcoded yeast open reading frame (MoBY-ORF) library, in which each gene is cloned into a centromere-based vector along with two unique oligonucleotide barcodes, was used to identify genes that, when mutated, confer drug resistance. In a previous study by the same research group,

theopalauamide was suggested to have a common molecular target with stichloroside (see Fig. 7), a marine-derived saponin: two metabolites showed similar chemical genetic profiles, and isolated mutant cells resistant to stichloroside were also resistant to theopalauamide despite their different chemical structures.[22] Using the MoBY-ORF complementation assay, the common resistant mutant was shown to have a mutation in *MVD1*, which encodes mevalonate pyrophosphate decarboxylase. This is an essential enzyme involved in an early step of the ergosterol biosynthesis pathway. The strategy of Yoshida and colleagues was based on the ORFeome overexpression strain collections, in which each ORF can be expressed in fission yeast cells.[195-196] They tested the sensitivity of ORFeome strains to TNM to obtain a chemical genomic profile. By comparing the chemical genomic profiles of TNM and reference compounds with known mechanisms of action, TNM was suggested to have a common target molecule with polyene macrolides, amphotericin B and nystatin. Lipid binding experiments using fluorescently-labeled TNMs (fTNMs, Fig. 25), disruption of liposomes by compounds, and yeast genetic analysis, were conducted to prove that TNMs target ergosterol in yeast.

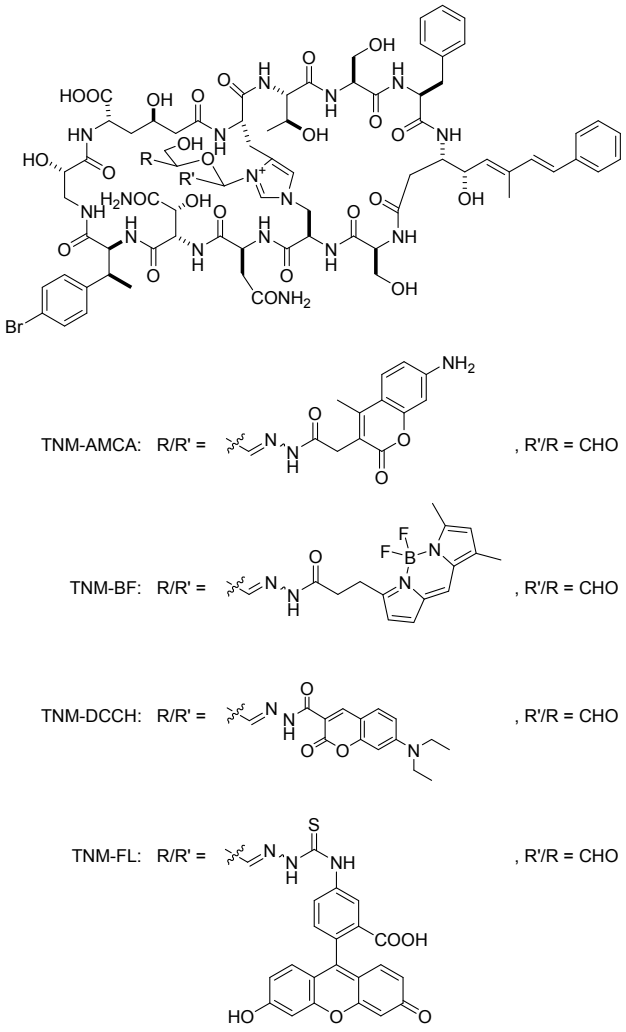


Fig. 25 Chemical structures of fluorescently labeled TNMs.

The mode of interaction of TNM with sterols at the molecular level was analyzed by surface plasmon resonance (SPR) experiments and ^{2}H NMR measurements, the latter of which was used for the AmB-sterol interaction analysis as described above. In the SPR experiments,[197] POPC liposomes containing cholesterol, ergosterol, or epicholesterol (3α-cholesterol) were immobilized on the SPR sensor chip, and the affinity of TNM-A to the liposomes was evaluated (Fig. 26). The results showed that TNM-A had strong affinity to both cholesterol- and ergosterol-containing membranes, whereas epicholesterol, which has a different 3-OH stereochemistry, did not enhance the membrane affinity of TNM-A (Fig. 26). These experiments confirmed that the peptide strictly recognizes the stereochemistry of the 3β-hydroxy group. The kinetic analyses revealed that the 3β-sterols markedly promote the initial binding of TNM-A to the membrane surfaces. The direct interaction between TNM-A and sterols was investigated

by solid-state ^{2}H NMR experiments, as with AmB.[197] As a result, 3-*d*-cholesterol and ergosterol gave broadened signals in the presence of TNM-A, while the doublet signal of 3-*d*-epicholesterol was not significantly affected by its presence. This demonstrated that TNM-A directly interacts with cholesterol and ergosterol in membranes, but has a much weaker interaction with epicholesterol. Unlike AmB, which recognizes and distinguishes the minute structural difference between ergosterol and cholesterol, TNM-A showed a small difference in affinity between both sterols. This suggested that TNM-A does not strictly distinguish the steroid skeleton or side chain structure, but mostly recognizes the 3β-OH moieties of sterols. Based on the results of SPR and solid state NMR experiments, TNM-A was suggested to recognize sterols in a shallow area of the lipid bilayer. In fact, TNM-A is assumed to have a hydrophobic cavity that can accommodate the sterol alicyclic part, including the 3β-OH group (Fig. 26).[198]

After binding to membrane sterols, TNM changes the membrane curvature and/or membrane fluidity. ^{31}P NMR experiments, dynamic light scattering (DLS) measurements, and confocal micrographs (Fig. 27) showed that TNM induces dynamical morphological change in membranes.[199] Taking into account that TNM-A does not form distinct pores in membranes,[197] a possible scenario of TNM's membrane activity is that it binds to the membrane surface and accumulates there through direct interaction with the 3β-OH moiety of sterols and consequently modifies the local membrane curvature. This results in dramatic membrane morphological changes and perturbations of membrane integrity (Fig. 27),[198-200] which ignites some biological events associated with the TNM-A activities. However, the fluidity of the membrane was shown to be important for the binding of TNM.[201] TNMs recognize cholesterol-containing liquid-disordered domains. In addition, phase separation was demonstrated by TNM-A in model lipid membranes (Fig. 27). Modulation of the membrane order was also observed in living cells following treatment with TNM-A, in which cells shrank considerably in a cholesterol-, cytoskeleton-, and energy-dependent manner. A mode of action study of TNM-A argued the importance of the membrane order, which is maintained by cholesterol, for proper cell morphogenesis.

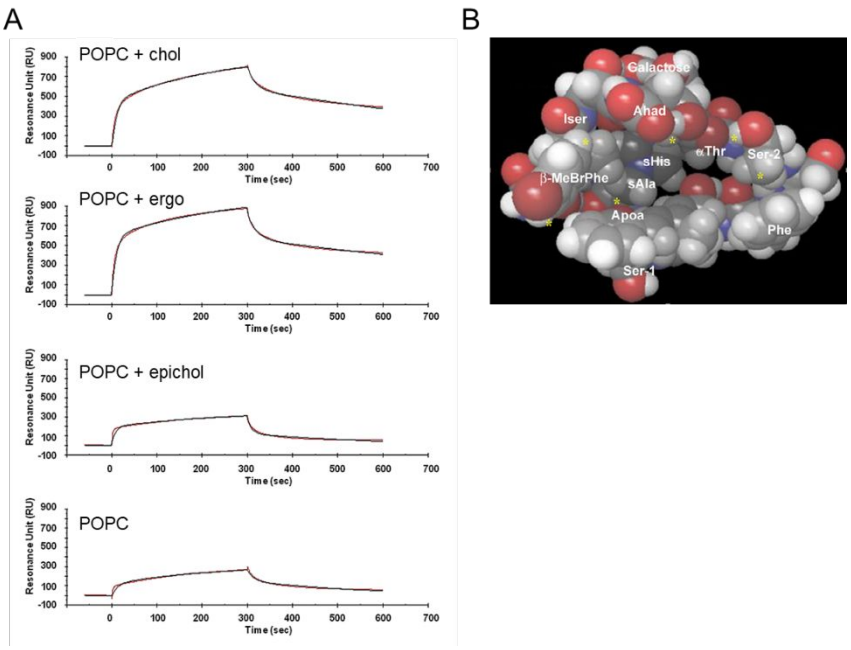


Fig. 26. Sterol binding of TNM-A. (A) SPR sensorgrams between TMN-A and sterol-containing POPC membranes. A total of 10 mol% of sterols were included in the POPC liposomes. (B) A hypothetical 3D structure of TNM-A that possesses a hydrophobic binding pocket or a cavity. The structure was generated from a conformational search using distance and dihedral angle constraints derived from experimental NOEs and ^{3}J-coupling data. Adapted with permission from refs. 197 and 198. Copyright 2017 American Chemical Society.

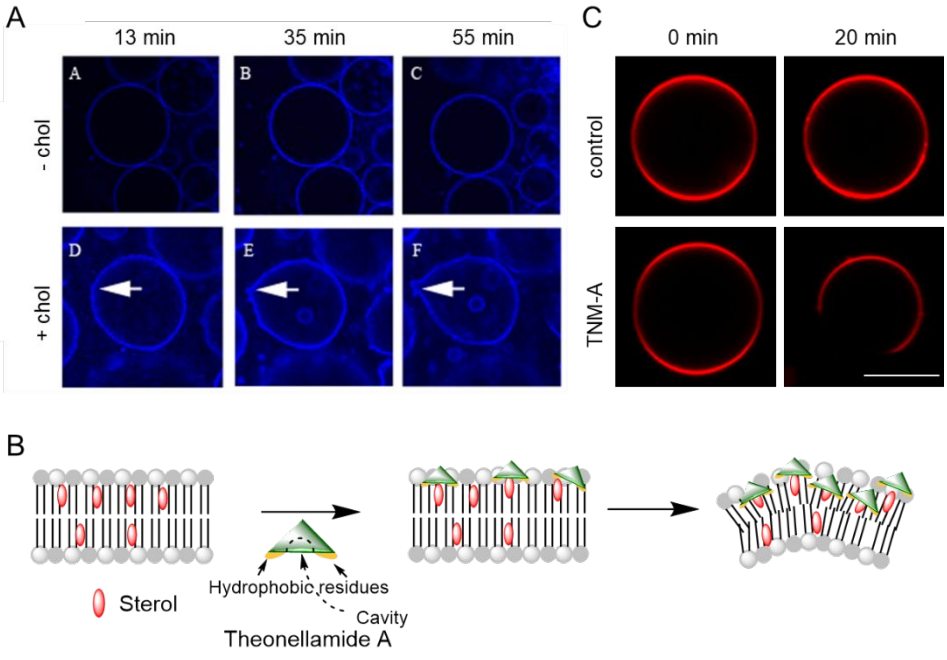


Fig. 27. Effect of TNM on the membrane structure. (A) Time-lapse confocal fluorescent microscopy images of sterol-free POPC GUVs (A–C) and cholesterol-containing POPC GUVs (D–F) after addition of 9:1 mol% TNM-A:TNM-DCCH to a final concentration of 20 μM. The arrows in the bottom images indicate the membrane protrusion induced by the peptide. (B) A possible mechanism of TNM-A-induced membrane morphological alteration. (C) Phase separation of lipid membranes by TNM-A. GUVs (DOPC/SM/Chol/Rh-DOPE, 24:25:50:1) were incubated with 1 μM TNM-A for 20 min, and confocal sections in the equatorial plane of GUVs are shown. Scale bar, 10 μm. Adapted from refs. 198, 199 and 201 with permission from the publisher.

TNMs induce ectopic accumulation of cell wall material in the fission yeast *Schizosaccharomyces pombe*. This was first inferred by chemical genomic analysis; GO analysis suggested that the mechanism of action partially overlaps with FK463, a 1,3-β-glucan synthase inhibitor.[21] In contrast to FK463, TNM induced overproduction of cell wall material, which counteracted FK463. Furthermore, a similar cell wall defect was observed after treatment of cells with heronamides.[72] The current understanding of cell wall overproduction is described in Section 5.2 and illustrated in Fig. 17.

fTNMs have been used as sterol markers in eukaryotic model organisms, budding yeast and fission yeast.[20-21, 202] Cholesterol in cultured mammalian cells and tissues can also be visualized under fluorescence microscopy.[203-206] It is noted that TNM-BF, a BODIPY-conjugated fTNM, is compatible for detecting cholesterol under electron microscopy.[207] Proteinous probes, such as perfringolysin O derivatives, can also detect sterols under electron microscopy.[52, 57] Comparative analysis using fTNMs and proteinous probes would identify novel sterol function because they show different membrane-binding properties.[52]

Currently, marine sponges are the sole source for theonellamide. Taking advantage of the abundance of the peptides isolated from marine sponges, biological and biophysical studies have been carried out. For further elucidation, such as studying molecular interactions between TNM and sterols at an atomic resolution, total synthesis of TNMs is required. TNM-inspired synthetic compounds with specific binding to fungal ergosterol would be promising candidates for drug development. However, the molecular mechanism underlying unique biological phenomena induced by TNMs have only been partially clarified. Deeper and comprehensive analysis will likely unveil other functions of membrane sterols.

5.6. Syringomycin E, forming an ion channel in lipid membranes.

Syringomycin E is a phytotoxin produced by the bacterium *Pseudomonas syringae* pv. syringae isolated from plants (Fig. 28).[208-209] The 3-Hydroxyacyl chain is conjugated through an amide bond in the serine residue, while this serine closes the depsipeptide ring by forming an ester bond. In this lipodepsipeptide, three positively charged amino acids, two hydrophobic amino acids and three hydroxylated amino acids are clustered.[210] This charge distribution may contribute to the molecular recognition between syringomycin E molecules for oligomerization, and between syringomycin E and lipid molecules for residing in the membrane. This molecule forms an asymmetric, voltage-dependent channel in lipid membranes.[211] The radii of the cis- and trans-openings were estimated to be 0.25–0.35 nm and 0.5–0.9 nm, respectively. This channel was proposed to be formed by at least six syringomycin E molecules,[212] and not to be constructed with cholesterol.[213] Sterols and sphingolipids were suggested to affect the formation of syringomycin E channels through changes in membrane environments.[214] As a consequence of the membrane binding of syringomycin E, efflux and influx of a series of monovalent and divalent cations and changes in membrane potential occurs.[215] The efficacy of this compound toward cells is altered by a mutation in ergosterol and sphingolipid biosynthesis, at least in yeast cells. Tolerance to syringomycin E has been reported in mutants defective in the C-5 desaturase of sterol,[216] hydroxylation of sphingolipids (Fig. 1),[217-218] synthesis of very long-chain fatty acids incorporated into sphingolipids,[219] or modification of the head group of the sphingolipids.[219] In addition, increasing the amount of sphingolipids conferred resistance to syringomycin E.[220] Despite the accumulating reports on chemical genetic interactions between syringomycin E and sphingolipids and ergosterol, the functional relationship between these membrane lipids and the syringomycin E channel is still not fully understood.

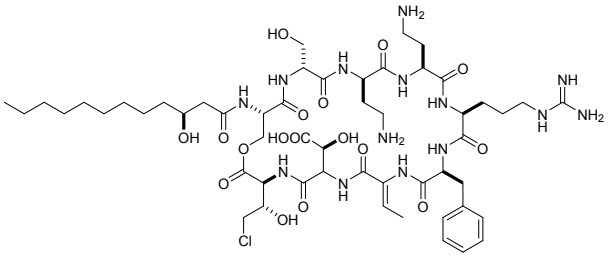


Fig. 28 Chemical structure of syringomycin E.

5.7. Amphidinol 3, favoring the 3β-sterol-containing membrane.

Amphidinols (AMs) are a class of polyhydroxyl polyene compounds isolated from dinoflagellates *Amphidinium* spp. Since the first member of this family was reported as a potent antifungal agent in 1991,[221] more than 20 amphidinols[222-232] and many related congeners, including certeraols,[233] lingshuiols,[234-235] karatungiols,[236] symbiopolyol,[237] luteophanols,[238] and amdigenol,[239] were isolated from the dinoflagellates. These compounds share a common structure with two tetrahydropyran rings linked by a carbon chain with a exomethylene group. Among amphidinols and their congeners, amphidinol 3 (AM3, Fig. 29) has the strongest hemolytic and antifungal activities. The stereochemistry of AM3 was first reported in 1999,[240] but revised several times. Only very recently did the revisions settled on the definite structure shown in Fig. 29.[241] AM3 forms membrane pores in a sterol-dependent manner,[242] which is thought to be responsible for its antifungal and hemolytic activities. However, its mode of interaction with sterol, as well as its mechanism of pore formation, are still not fully understood.

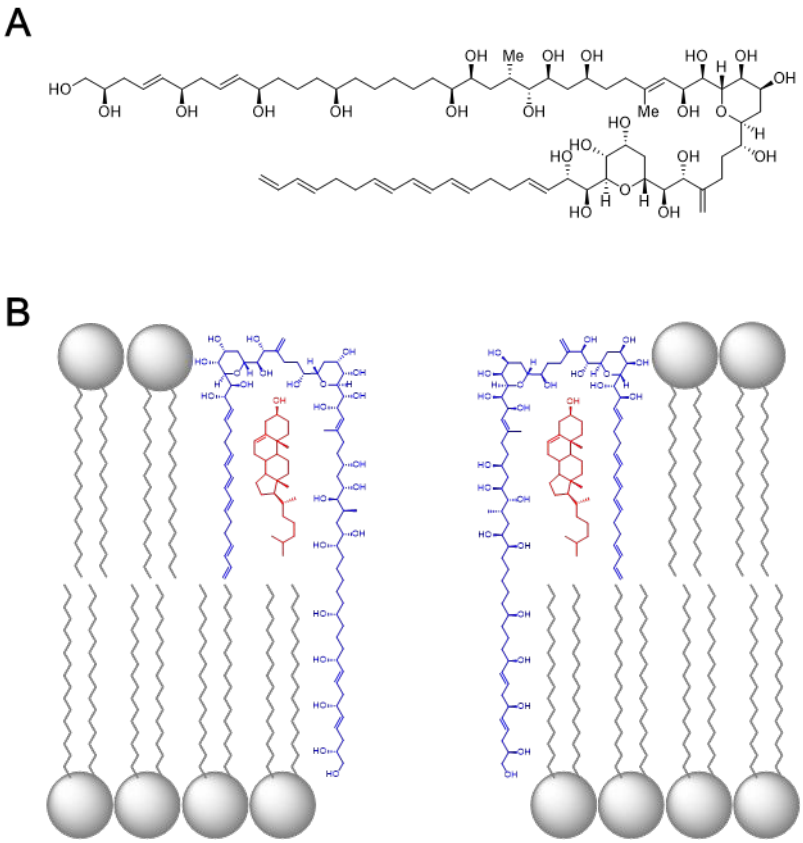


Fig. 29. Structures of amphidinol 3 (AM3). Chemical structure of AM3 (A) and a model of the AM3 barrel-stave channel, which incorporates cholesterol (B), are shown. Adapted with permission from ref. 243. Copyright 2014 American Chemical Society.

The specific interaction of AM3 with sterols was investigated by observing calcein dye leakage from liposomes containing cholesterol, ergosterol, and epicholesterol.[243] AM3 showed a strong dye leakage activity in ergosterol- or cholesterol-containing liposomes, while epicholesterol did not promote AM3-induced dye leakage at all. The SPR and solid-state ^{2}H NMR measurements, which were also used for AmB and TNM-A, as described above, clearly showed that AM3 directly interacts with cholesterol and ergosterol in membranes, but has much weaker interaction with epicholesterol.[243] These experiments demonstrated that AM3 strictly recognizes the stereochemistry of the 3-OH group and exerts its pore forming activity. Although the sterol selectivity of AM3 between ergosterol and cholesterol was less clear in the dye leakage and the SPR experiments, recent channel-current recording experiments showed a significantly larger activity of AM3 in an ergosterol-containing membrane,[244] which may suggest that the polyene portion of AM3 recognizes the sterol hydrophobic region to some extent.

Although AM3 forms distinct membrane pores in a sterol-dependent manner, it is still controversial whether the pore is a toroidal or barrel-stave type. In previous reports, membrane permeabilization of AM3 was assumed to follow a toroidal pore model in which the lipid monolayer bends continuously from the outer to the inner leaflets of membrane because the membrane permeabilizing activity of AM3 was less sensitive to the membrane thickness. [242, 245-246] However, although toroidal pore formation should give isotropic signals in solid-state ^{31}P NMR spectra, no spectral changes were observed in cholesterol- or ergosterol-containing POPC liposomes, even in the presence of a high AM3 molar ratio.[243] This suggests that the mechanism of AM3 action may be accounted for by a transmembrane barrel-stave pore. It was previously reported that AM3 takes a hairpin-like conformation with a relatively rigid turn structure at the middle region, including the two tetrahydropyran rings.[245-246] Assuming that the turned region, which is conserved among other amphidinols, recognizes the 3β-OH group of sterols via hydrogen bonds, a hypothetical barrel-stave model was proposed to explain the membrane permeabilization activity of AM3 interacting with sterol molecules (Fig. 29).[243] In this model, the AM3 polyol chain penetrates the membrane and forms the hydrophilic inner pore, while the polyene part is lined with the steroidal skeleton and may be responsible for the ability to discern cholesterol from ergosterol. In a recent channel-

recording experiment,[244] however, AM3 was shown to form both barrel-stave type transmembrane channels and toroidal-like pores in a concentration dependent manner; at a lower concentration of AM3, a barrel-stave pore is formed, while at a higher concentration, toroidal pore formation prevails. Although the interaction model proposed for the barrel-stave pore formation of AM3 may partly hold true for the toroidal pore, the AM3 polyol chain does not likely penetrate the membrane but exists on the membrane surface and increases the membrane local curvature to facilitate the toroidal pore formation.[244]

6. Conclusion and future directions

Lipids are the major components of the cell membrane, and the number of lipid species is large, being estimated around 180,000.[29] Lipidomics analyses using LCMS have enabled quantitative and comprehensive examination of lipids,[31-32] thus there is a saturation of structural information, i.e. new lipids with unexpected chemical structures are rarely discovered. In contrast, it is functional analysis that now requires new methods and instruments. Natural products have and will directly contribute to this field as many unique compounds are able to recognize lipids with high affinity and specificity. In fact some are used as drugs and research tools already.

We have reviewed a representative but not exhaustive part of the research on natural products that target eukaryotic membrane lipids. The number of compounds that target lipids is likely much larger than currently known. For example, more than 1000 saponins have been reported, only some of which have been examined in their sterol binding ability. Curiously, their sterol specificity is affected by minor structural differences of the aglycon and the sugar portion.[82] In the case of cyclotides, more than 9000 species were estimated in the family of Violaceae.[247] Such a structural variety may display unique modes of lipid recognition and novel biological activities.

Ergosterol is the traditional drug target because this molecule is specifically distributed in fungi and essential for their growth. Polyene antibiotics are used as pharmaceutical agents, although they have limitations in their use owing to side effects. A current interaction model (Fig. 12) should be used to design new molecules with higher efficacy and pharmacokinetic properties, and lower toxicity. To achieve this goal, novel methods for synthesizing complex natural products are required: chemical synthesis, biosynthesis, and hybrids of these two; even in silico simulation methods can be used. In addition to ergosterol, sphingolipids unique to fungi and PE on the surface of the tumor vascular endothelium are drug target candidates.[28, 74] New molecules targeting these lipids await discovery in the ever expanding chemical space covered by natural products.

In this article, we classified the membrane-targeting natural products into three (types A–C), based on their lipid-binding modes (Fig. 6). However, in addition to molecules belonging to types A–C, which exhibit their biological activity at the membrane, there is a significant number of membrane-residing molecules. These include lipidated amphiphilic molecules, such as mycobactin produced by *Mycobacterium tuberculosis*,[248-249] and small lipophilic molecules, such as 5aTHQs produced by *Streptomyces nigrescens* when cultured with *Tsukamurella pulmonisin*.[73, 250-251] Further exploration will provide discoveries of novel natural products that show unique biological functions by residing in/on the membrane.

A central question is why membrane-targeting molecules are widely distributed in the natural world. Chemists know that amphiphilic nature becomes troublesome in test tubes in the laboratory. Amphiphilic molecules exhibit bumping during evaporation, separating minor congeners based on the variation of the acyl chain is difficult, and aggregation gives poor signals in the NMR spectra. How do organisms, who produce such molecules, recognize, regulate and use these troublesome natural products? Understanding the physiological roles of natural products that target or reside in/on the membrane should be fun, and be beneficial for controlling organisms such as pathogen and weeds, and human diseases.

7. Conflict of interest

There are no conflicts to declare.

8. Acknowledgements

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9. References

[1] M. Feher, J. M. Schmidt, *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 218-227.

[2] D. J. Newman, G. M. Cragg, *J. Nat. Prod.* **2016**, *79*, 629-661.

[3] E. E. Carlson, *ACS Chem. Biol.* **2010**, *5*, 639-653.

[4] B. M. Dunyak, J. E. Gestwicki, *J. Med. Chem.* **2016**, *59*, 9622-9644.

[5] S. L. Schreiber, *Science* **1991**, *251*, 283-287.

[6] I. Mnif, D. Ghribi, *Biopolymers* **2015**, *104*, 129-147.

[7] J. M. Raaijmakers, I. De Bruijn, O. Nybroe, M. Ongena, *FEMS Microbiol. Rev.* **2010**, *34*, 1037-1062.

[8] Y. Bahrami, C. M. Franco, *Mar. Drugs* **2016**, *14*.

[9] G. Xiao, X. Shao, D. Zhu, B. Yu, *Nat. Prod. Rep.* **2019**, *36*, 769-787.

[10] J. P. Vincken, L. Heng, A. de Groot, H. Gruppen, *Phytochemistry* **2007**, *68*, 275-297.

[11] J. M. Augustin, V. Kuzina, S. B. Andersen, S. Bak, *Phytochemistry* **2011**, *72*, 435-457.

[12] G. Caulier, P. Flammang, P. Gerbaux, I. Eeckhaut, *Sci. Rep.* **2013**, *3*, 2639.

[13] C. R. Pye, M. J. Bertin, R. S. Lokey, W. H. Gerwick, R. G. Linington, *Proc. Natl. Acad. Sci. U.S.A.* **2017**, *114*, 5601-5606.

[14] S. Ziegler, V. Pries, C. Hedberg, H. Waldmann, *Angew. Chem. Int. Ed.* **2013**, *52*, 2744-2792.

[15] N. Kanoh, *Nat. Prod. Rep.* **2016**, *33*, 709-718.

[16] A. M. Piggott, P. Karuso, *Nat. Prod. Rep.* **2016**, *33*, 626-636.

[17] Y. Ohya, Y. Kimori, H. Okada, S. Ohnuki, *Mol. Biol. Cell* **2015**, *26*, 3920-3925.

[18] K. Andrusiak, J. S. Piotrowski, C. Boone, *Bioorg. Med. Chem.* **2012**, *20*, 1952-1960.

[19] M. Muroi, Y. Futamura, H. Osada, *Nat. Prod. Rep.* **2016**, *33*, 621-625.

[20] C. H. Ho, L. Magtanong, S. L. Barker, D. Gresham, S. Nishimura, P. Natarajan, J. L. Koh, J. Porter, C. A. Gray, R. J. Andersen, G. Giaever, C. Nislow, B. Andrews, D. Botstein, T. R. Graham, M. Yoshida, C. Boone, *Nat. Biotechnol.* **2009**, *27*, 369-377.

[21] S. Nishimura, Y. Arita, M. Honda, K. Iwamoto, A. Matsuyama, A. Shirai, H. Kawasaki, H. Kakeya, T. Kobayashi, S. Matsunaga, M. Yoshida, *Nat. Chem. Biol.* **2010**, *6*, 519-526.

[22] A. B. Parsons, A. Lopez, I. E. Givoni, D. E. Williams, C. A. Gray, J. Porter, G. Chua, R. Sopko, R. L. Brost, C. H. Ho, J. Wang, T. Ketela, C. Brenner, J. A. Brill, G. E. Fernandez, T. C. Lorenz, G. S. Payne, S. Ishihara, Y. Ohya, B. Andrews, T. R. Hughes, B. J. Frey, T. R. Graham, R. J. Andersen, C. Boone, *Cell* **2006**, *126*, 611-625.

[23] R. M. Epand, C. Walker, R. F. Epand, N. A. Magarvey, *Biochim. Biophys. Acta* **2016**, *1858*, 980-987.

[24] D. Ciumac, H. Gong, X. Hu, J. R. Lu, *J. Colloid. Interface Sci.* **2019**, *537*, 163-185.

[25] A. Naito, N. Matsumori, A. Ramamoorthy, *Biochim. Biophys. Acta Gen. Subj.* **2018**, *1862*, 307-323.

[26] A. Marquette, B. Bechinger, *Biomolecules* **2018**, *8*.

[27] J. T. Hannich, K. Umebayashi, H. Riezman, *Cold Spring Harb. Perspect. Biol.* **2011**, *3*.

[28] J. T. Marques, H. S. Marinho, R. F. M. de Almeida, *Prog. Lipid Res.* **2018**, *71*, 18-42.

[29] L. Yetukuri, K. Ekroos, A. Vidal-Puig, M. Oresic, *Mol. Biosyst.* **2008**, *4*, 121-127.

[30] C. S. Ejsing, J. L. Sampaio, V. Surendranath, E. Duchoslav, K. Ekroos, R. W. Klemm, K. Simons, A. Shevchenko, *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 2136-2141.

[31] K. Yang, X. Han, *Trends Biochem. Sci.* **2016**, *41*, 954-969.

[32] X. Han, *Nat. Rev. Endocrinol.* **2016**, *12*, 668-679.

[33] T. J. Beeler, D. Fu, J. Rivera, E. Monaghan, K. Gable, T. M. Dunn, *Mol. Gen. Genet.* **1997**, *255*, 570-579.

[34] K. Umebayashi, A. Nakano, *J. Cell Biol.* **2003**, *161*, 1117-1131.

[35] Z. Jiang, X. Zhou, M. Tao, F. Yuan, L. Liu, F. Wu, X. Wu, Y. Xiang, Y. Niu, F. Liu, C. Li, R. Ye, B. Byeon, Y. Xue, H. Zhao, H. N. Wang, B. M. Crawford, D. M. Johnson, C. Hu, C. Pei, W. Zhou, G. B. Swift, H. Zhang, T. Vo-Dinh, Z. Hu, J. N. Siedow, Z. M. Pei, *Nature* **2019**, *572*, 341-346.

[36] J. Gronnier, V. Germain, P. Gouguet, J. L. Cacas, S. Mongrand, *Plant Signal. Behav.* **2016**, *11*, e1152438.

[37] A. L. Munn, A. Heese-Peck, B. J. Stevenson, H. Pichler, H. Riezman, *Mol. Biol. Cell* **1999**, *10*, 3943-3957.

[38] S. Mahammad, I. Parmryd, *Methods Mol. Biol.* **2015**, *1232*, 91-102.

[39] B. Mesmin, F. R. Maxfield, *Biochim. Biophys. Acta* **2009**, *1791*, 636-645.

[40] Y. Yang, M. Lee, G. D. Fairn, *J. Biol. Chem.* **2018**, *293*, 6230-6240.

[41] E. Sezgin, I. Levental, S. Mayor, C. Eggeling, *Nat. Rev. Mol. Cell Biol.* **2017**, *18*, 361-374.

[42] T. Yamaji, K. Hanada, *Traffic* **2015**, *16*, 101-122.

[43] K. Segawa, S. Nagata, *Trends Cell Biol.* **2015**, *25*, 639-650.

[44] P. G. Pentchev, *Biochim. Biophys. Acta* **2004**, *1685*, 3-7.

[45] S. R. Pfeffer, *J. Biol. Chem.* **2019**, *294*, 1706-1709.

[46] D. Lingwood, K. Simons, *Science* **2010**, *327*, 46-50.

[47] A. Kusumi, T. A. Tsunoyama, K. M. Hirosawa, R. S. Kasai, T. K. Fujiwara, *Nat. Chem. Biol.* **2014**, *10*, 524-532.

[48] M. Kinoshita, K. G. Suzuki, N. Matsumori, M. Takada, H. Ano, K. Morigaki, M. Abe,

A. Makino, T. Kobayashi, K. M. Hirosawa, T. K. Fujiwara, A. Kusumi, M. Murata, *J. Cell Biol.* **2017**, *216*, 1183-1204.

[49] R. Ishitsuka, S. B. Sato, T. Kobayashi, *J. Biochem.* **2005**, *137*, 249-254.

[50] A. Moriyama, N. Katagiri, S. Nishimura, N. Takahashi, H. Kakeya, *Sci. Rep.* **2015**, *5*, 17427.

[51] G. Gimpl, K. Gehrig-Burger, *Steroids* **2011**, *76*, 216-231.

[52] T. Kishimoto, R. Ishitsuka, T. Kobayashi, *Biochim. Biophys. Acta* **2016**, *1861*, 812-829.

[53] J. F. Frisz, K. Lou, H. A. Klitzing, W. P. Hanafin, V. Lizunov, R. L. Wilson, K. J. Carpenter, R. Kim, I. D. Hutcheon, J. Zimmerberg, P. K. Weber, M. L. Kraft, *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, E613-622.

[54] J. Ando, M. Kinoshita, J. Cui, H. Yamakoshi, K. Dodo, K. Fujita, M. Murata, M. Sodeoka, *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112*, 4558-4563.

[55] F. Hullin-Matsuda, A. Makino, M. Murate, T. Kobayashi, *Biochimie* **2016**, *130*, 81-90.

[56] H. Shogomori, T. Kobayashi, *Biochim. Biophys. Acta* **2008**, *1780*, 612-618.

[57] Y. Ohno-Iwashita, Y. Shimada, M. Hayashi, M. Iwamoto, S. Iwashita, M. Inomata, *Subcell. Biochem.* **2010**, *51*, 597-621.

[58] W. Drabikow, E. Lagwinsk, M. G. Sarzala, *Biochim. Biophys. Acta* **1973**, *291*, 61-70.

[59] F. R. Maxfield, D. Wustner, *Methods Cell Biol.* **2012**, *108*, 367-393.

[60] M. Heidary, A. D. Khosravi, S. Khoshnood, M. J. Nasiri, S. Soleimani, M. Goudarzi, *J. Antimicrob. Chemother.* **2018**, *73*, 1-11.

[61] S. D. Taylor, M. Palmer, *Bioorg. Med. Chem.* **2016**, *24*, 6253-6268.

[62] H. Hamamoto, M. Urai, K. Ishii, J. Yasukawa, A. Paudel, M. Murai, T. Kaji, T. Kuranaga, K. Hamase, T. Katsu, J. Su, T. Adachi, R. Uchida, H. Tomoda, M. Yamada, M. Souma, H. Kurihara, M. Inoue, K. Sekimizu, *Nat. Chem. Biol.* **2015**, *11*, 127-133.

[63] M. Santiago, W. Lee, A. A. Fayad, K. A. Coe, M. Rajagopal, T. Do, F. Hennessen, V. Srisuknimit, R. Muller, T. C. Meredith, S. Walker, *Nat. Chem. Biol.* **2018**, *14*, 601-608.

[64] L. Ostrosky-Zeichner, A. Casadevall, J. N. Galgiani, F. C. Odds, J. H. Rex, *Nat. Rev. Drug Discov.* **2010**, *9*, 719-727.

[65] F. C. Odds, A. J. Brown, N. A. Gow, *Trends Microbiol.* **2003**, *11*, 272-279.

[66] A. F. G. Cicero, M. Landolfo, F. Ventura, C. Borghi, *Expert Opin. Pharmacother.* **2019**, *20*, 1277-1288.

[67] L. Ostrosky-Zeichner, K. A. Marr, J. H. Rex, S. H. Cohen, *Clin. Infect. Dis.* **2003**, *37*, 415-425.

[68] S. Shimada, *Science* **1969**, *163*, 1462.

[69] J. B. Anderson, *Nat. Rev. Microbiol.* **2005**, *3*, 547-556.

[70] D. Hughes, D. I. Andersson, *Nat. Rev. Genet.* **2015**, *16*, 459-471.

[71] B. M. Vincent, A. K. Lancaster, R. Scherz-Shouval, L. Whitesell, S. Lindquist, *PLoS Biol.* **2013**, *11*, e1001692.

[72] R. Sugiyama, S. Nishimura, N. Matsumori, Y. Tsunematsu, A. Hattori, H. Kakeya, *J. Am. Chem. Soc.* **2014**, *136*, 5209-5212.

[73] R. Sugiyama, S. Nishimura, T. Ozaki, S. Asamizu, H. Onaka, H. Kakeya, *Org. Lett.* **2015**, *17*, 1918-1921.

[74] J. H. Stafford, P. E. Thorpe, *Neoplasia* **2011**, *13*, 299-308.

[75] S. Ran, A. Downes, P. E. Thorpe, *Cancer. Res.* **2002**, *62*, 6132-6140.

[76] S. Ran, J. He, X. Huang, M. Soares, D. Scothorn, P. E. Thorpe, *Clin. Cancer Res.* **2005**, *11*, 1551-1562.

[77] C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, *Adv. Drug Deliv. Rev.* **2001**, *46*, 3-26.

[78] L. Cascales, S. T. Henriques, M. C. Kerr, Y. H. Huang, M. J. Sweet, N. L. Daly, D. J. Craik, *J. Biol. Chem.* **2011**, *286*, 36932-36943.

[79] S. T. Henriques, Y. H. Huang, K. J. Rosengren, H. G. Franquelim, F. A. Carvalho, A. Johnson, S. Sonza, G. Tachedjian, M. A. Castanho, N. L. Daly, D. J. Craik, *J. Biol. Chem.* **2011**, *286*, 24231-24241.

[80] N. Frenkel, A. Makky, I. R. Sudji, M. Wink, M. Tanaka, *J. Phys. Chem. B* **2014**, *118*, 14632-14639.

[81] E. A. Keukens, T. de Vrije, C. H. Fabrie, R. A. Demel, W. M. Jongen, B. de Kruijff, *Biochim. Biophys. Acta* **1992**, *1110*, 127-136.

[82] E. A. Keukens, T. de Vrije, C. van den Boom, P. de Waard, H. H. Plasman, F. Thiel, V. Chupin, W. M. Jongen, B. de Kruijff, *Biochim. Biophys. Acta* **1995**, *1240*, 216-228.

[83] H. Tsuchiya, *Molecules* **2017**, *22*.

[84] T. Hamada, S. Matsunaga, G. Yano, N. Fusetani, *J. Am. Chem. Soc.* **2005**, *127*, 110-118.

[85] T. Hamada, S. Matsunaga, M. Fujiwara, K. Fujita, H. Hirota, R. Schmucki, P. Guntert, N. Fusetani, *J. Am. Chem. Soc.* **2010**, *132*, 12941-12945.

[86] Y. Matsuki, M. Iwamoto, K. Mita, K. Shigemi, S. Matsunaga, S. Oiki, *J. Am. Chem. Soc.* **2016**, *138*, 4168-4177.

[87] A. Hayata, H. Itoh, M. Inoue, *J. Am. Chem. Soc.* **2018**, *140*, 10602-10611.

[88] S. B. Zotchev, *Curr. Med. Chem.* **2003**, *10*, 211-223.

[89] Z. A. Kanafani, J. R. Perfect, *Clin. Infect. Dis.* **2008**, *46*, 120-128.

[90] B. de Kruijff, R. A. Demel, *Biochim. Biophys. Acta* **1974**, *339*, 57-70.

[91] J. Bolard, *Biochim. Biophys. Acta* **1986**, *864*, 257-304.

[92] Y. M. te Welscher, H. H. ten Napel, M. M. Balague, C. M. Souza, H. Riezman, B. de Kruijff, E. Breukink, *J. Biol. Chem.* **2008**, *283*, 6393-6401.

[93] Y. M. te Welscher, M. R. van Leeuwen, B. de Kruijff, J. Dijksterhuis, E. Breukink, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 11156-11159.

[94] T. M. Anderson, M. C. Clay, A. G. Cioffi, K. A. Diaz, G. S. Hisao, M. D. Tuttle, A. J. Nieuwkoop, G. Comellas, N. Maryum, S. Wang, B. E. Uno, E. L. Wildeman, T. Gonen, C. M. Rienstra, M. D. Burke, *Nat. Chem. Biol.* **2014**, *10*, 400-406.

[95] K. C. Gray, D. S. Palacios, I. Dailey, M. M. Endo, B. E. Uno, B. C. Wilcock, M. D. Burke, *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 2234-2239.

[96] N. Matsumori, K. Tahara, H. Yamamoto, A. Morooka, M. Doi, T. Oishi, M. Murata, *J. Am. Chem. Soc.* **2009**, *131*, 11855-11860.

[97] Y. Nakagawa, Y. Umegawa, T. Takano, H. Tsuchikawa, N. Matsumori, M. Murata, *Biochemistry* **2014**, *53*, 3088-3094.

[98] Y. Nakagawa, Y. Umegawa, K. Nonomura, N. Matsushita, T. Takano, H. Tsuchikawa, S. Hanashima, T. Oishi, N. Matsumori, M. Murata, *Biochemistry* **2015**, *54*, 303-312.

[99] Y. Umegawa, Y. Nakagawa, K. Tahara, H. Tsuchikawa, N. Matsumori, T. Oishi, M. Murata, *Biochemistry* **2012**, *51*, 83-89.

[100] Y. Nakagawa, Y. Umegawa, N. Matsushita, T. Yamamoto, H. Tsuchikawa, S. Hanashima, T. Oishi, N. Matsumori, M. Murata, *Biochemistry* **2016**, *55*, 3392-3402.

[101] B. Venegas, J. Gonzalez-Damian, H. Celis, I. Ortega-Blake, *Biophys. J* **2003**, *85*, 2323-2332.

[102] T. Gullion, J. Schaefer, *J. Magn. Reson.* **1989**, *81*, 196-200.

[103] N. Matsumori, Y. Umegawa, T. Oishi, M. Murata, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3565-3567.

[104] M. Nazare, H. Waldmann, *Angew. Chem. Int. Ed.* **2000**, *39*, 1125-1128.

[105] C. Thawai, P. Kittakoop, S. Tanasupawat, K. Suwanborirux, K. Sriklung, Y. Thebtaranonth, *Chem. Biodivers.* **2004**, *1*, 640-645.

[106] E. J. Skellam, A. K. Stewart, W. K. Strangman, J. L. Wright, *J. Antibiot. (Tokyo)* **2013**, *66*, 431-441.

[107] D. C. Oh, M. Poulsen, C. R. Currie, J. Clardy, *Org. Lett.* **2011**, *13*, 752-755.

[108] S. S. Mitchell, B. Nicholson, S. Teisan, K. S. Lam, B. C. Potts, *J. Nat. Prod.* **2004**, *67*, 1400-1402.

[109] D. W. Udwary, L. Zeigler, R. N. Asolkar, V. Singan, A. Lapidus, W. Fenical, P. R. Jensen, B. S. Moore, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 10376-10381.

[110] C. J. Schulze, M. S. Donia, J. L. Siqueira-Neto, D. Ray, J. A. Raskatov, R. E. Green, J. H. McKerrow, M. A. Fischbach, R. G. Linington, *ACS Chem. Biol.* **2015**, *10*, 2373-2381.

[111] K. Shindo, M. Kamishohara, A. Odagawa, M. Matsuoka, H. Kawai, *J. Antibiot.* **1993**, *46*, 1076-1081.

[112] Y. Futamura, R. Sawa, Y. Umezawa, M. Igarashi, H. Nakamura, K. Hasegawa, M. Yamasaki, E. Tashiro, Y. Takahashi, Y. Akamatsu, M. Imoto, *J. Am. Chem. Soc.* **2008**, *130*, 1822-1823.

[113] D. Schulz, J. Nachtigall, U. Geisen, H. Kalthoff, J. F. Imhoff, H. P. Fiedler, R. D. Sussmuth, *J. Antibiot. (Tokyo)* **2012**, *65*, 369-372.

[114] C. Beemelmanns, T. R. Ramadhar, K. H. Kim, J. L. Klassen, S. Cao, T. P. Wyche, Y. Hou, M. Poulsen, T. S. Bugni, C. R. Currie, J. Clardy, *Org. Lett.* **2017**, *19*, 1000-1003.

[115] S. Hoshino, M. Okada, T. Wakimoto, H. Zhang, F. Hayashi, H. Onaka, I. Abe, *J. Nat. Prod.* **2015**, *78*, 3011-3017.

[116] D. K. Derewacz, B. C. Covington, J. A. McLean, B. O. Bachmann, *ACS Chem. Biol.* **2015**, *10*, 1998-2006.

[117] M. Otsuka, M. Fujita, Y. Matsushima, T. Eguchi, K. Shindo, K. Kakinuma, *Tetrahedron* **2000**, *56*, 8281-8286.

[118] Z. J. Low, L. M. Pang, Y. Ding, Q. W. Cheang, K. Le Mai Hoang, H. Thi Tran, J. Li, X. W. Liu, Y. Kanagasundaram, L. Yang, Z. X. Liang, *Sci. Rep.* **2018**, *8*, 1594.

[119] M. Takaishi, F. Kudo, T. Eguchi, *Org. Lett.* **2012**, *14*, 4591-4593.

[120] H. Jorgensen, K. F. Degnes, H. Sletta, E. Fjaervik, A. Dikiy, L. Herfindal, P. Bruheim, G. Klinkenberg, H. Bredholt, G. Nygard, S. O. Doskeland, T. E. Ellingsen, S. B. Zotchev, *Chem. Biol.* **2009**, *16*, 1109-1121.

[121] H. Jorgensen, K. F. Degnes, A. Dikiy, E. Fjaervik, G. Klinkenberg, S. B. Zotchev, *Appl. Environ. Microbiol.* **2010**, *76*, 283-293.

[122] Y. Zhu, W. Zhang, Y. Chen, C. Yuan, H. Zhang, G. Zhang, L. Ma, Q. Zhang, X. Tian, S. Zhang, C. Zhang, *Chembiochem* **2015**, *16*, 2086-2093.

[123] K. Kojiri, S. Nakajima, H. Suzuki, H. Kondo, H. Suda, *J. Antibiot.* **1992**, *45*, 868-874.

[124] I. Takahashi, Y. Oda, Y. Nishiie, K. Ochiai, T. Mizukami, *J. Antibiot. (Tokyo)* **1997**, *50*, 186-188.

[125] R. Raju, A. M. Piggott, M. M. Conte, R. J. Capon, *Org. Biomol. Chem.* **2010**, *8*, 4682-4689.

[126] W. Zhang, S. Li, Y. Zhu, Y. Chen, Y. Chen, H. Zhang, G. Zhang, X. Tian, Y. Pan, S. Zhang, W. Zhang, C. Zhang, *J. Nat. Prod.* **2014**, *77*, 388-391.

[127] R. Sugiyama, S. Nishimura, H. Kakeya, *Tetrahedron Lett.* **2013**, *54*, 1531-1533.

[128] N. Kanoh, S. Itoh, K. Fujita, K. Sakanishi, R. Sugiyama, Y. Terajima, Y. Iwabuchi, S. Nishimura, H. Kakeya, *Chemistry* **2016**, *22*, 8586-8595.

[129] P. Yu, A. Patel, K. N. Houk, *J. Am. Chem. Soc.* **2015**, *137*, 13518-13523.

[130] T. J. Booth, S. Alt, R. J. Capon, B. Wilkinson, *Chem. Commun.* **2016**, *52*, 6383-6386.

[131] N. Ding, L. Han, Y. Jiang, G. D. Li, Z. H. Zhen, B. X. Cao, P. P. Guan, Y. Mu, B. Lin, X. S. Huang, *Rsc Adv.* **2018**, *8*, 17121-17131.

[132] K. Komiyama, K. Iwasaki, M. Miura, H. Yamamoto, Y. Nozawa, I. Umezawa, *J. Antibiot. (Tokyo)* **1985**, *38*, 1614-1616.

[133] M. Nazare, H. Waldmann, *Angew. Chem. Int. Ed.* **2000**, *39*, 1125-1128.

[134] T. Kawahara, T. Fujiwara, N. Kagaya, K. Shin-Ya, *J. Antibiot. (Tokyo)* **2018**, *71*, 390-392.

[135] M. Hampsey, *Yeast* **1997**, *13*, 1099-1133.

[136] T. Iwaki, H. Iefuji, Y. Hiraga, A. Hosomi, T. Morita, Y. Giga-Hama, K. Takegawa, *Microbiology* **2008**, *154*, 830-841.

[137] K. Simons, W. L. Vaz, *Annu. Rev. Biophys. Biomol. Struct.* **2004**, *33*, 269-295.

[138] J. Huang, G. W. Feigenson, *Biophys. J.* **1999**, *76*, 2142-2157.

[139] H. M. McConnell, A. Radhakrishnan, *Biochim. Biophys. Acta* **2003**, *1610*, 159-173.

[140] K. Sakanishi, S. Itoh, R. Sugiyama, S. Nishimura, H. Kakeya, Y. Iwabuchi, N. Kanoh, *Eur. J. Org. Chem.* **2014**, *2014*, 1376-1380.

[141] K. Fujita, R. Sugiyama, S. Nishimura, N. Ishikawa, M. A. Arai, M. Ishibashi, H. Kakeya, *J. Nat. Prod.* **2016**, *79*, 1877-1880.

[142] J. C. Cortes, J. Ishiguro, A. Duran, J. C. Ribas, *J. Cell Sci.* **2002**, *115*, 4081-4096.

[143] M. Arellano, A. Duran, P. Perez, *EMBO J.* **1996**, *15*, 4584-4591.

[144] A. Feoktistova, P. Magnelli, C. Abeijon, P. Perez, R. L. Lester, R. C. Dickson, K. L. Gould, *Genetics* **2001**, *158*, 1397-1411.

[145] G. Shui, X. L. Guan, C. P. Low, G. H. Chua, J. S. Goh, H. Yang, M. R. Wenk, *Mol. Biosyst.* **2010**, *6*, 1008-1017.

[146] J. Liu, X. Tang, H. Wang, S. Oliferenko, M. K. Balasubramanian, *Mol. Biol. Cell* **2002**, *13*, 989-1000.

[147] Y. Nishiyama, T. Ohmichi, S. Kazami, H. Iwasaki, K. Mano, Y. Nagumo, F. Kudo, S. Ichikawa, Y. Iwabuchi, N. Kanoh, T. Eguchi, H. Osada, T. Usui, *Biosci. Biotechnol. Biochem.* **2016**, *80*, 902-910.

[148] J. H. Wong, M. Alfatah, K. W. Kong, S. Hoon, W. L. Yeo, K. C. Ching, C. Jie Hui Goh, M. M. Zhang, Y. H. Lim, F. T. Wong, P. Arumugam, *PloS one* **2019**, *14*, e0218189.

[149] A. Okesli, L. E. Cooper, E. J. Fogle, W. A. van der Donk, *J. Am. Chem. Soc.* **2011**, *133*, 13753-13760.

[150] C. Chatterjee, M. Paul, L. Xie, W. A. van der Donk, *Chem. Rev.* **2005**, *105*, 633-684.

[151] D. A. Phoenix, F. Harris, M. Mura, S. R. Dennison, *Prog. Lipid Res.* **2015**, *59*, 26-37.

[152] K. Iwamoto, T. Hayakawa, M. Murate, A. Makino, K. Ito, T. Fujisawa, T. Kobayashi, *Biophys. J.* **2007**, *93*, 1608-1619.

[153] G. Machaidze, A. Ziegler, J. Seelig, *Biochemistry* **2002**, *41*, 1965-1971.

[154] K. Wakamatsu, S. Y. Choung, T. Kobayashi, K. Inoue, T. Higashijima, T. Miyazawa, *Biochemistry* **1990**, *29*, 113-118.

[155] K. Hosoda, M. Ohya, T. Kohno, T. Maeda, S. Endo, K. Wakamatsu, *Journal of biochemistry* **1996**, *119*, 226-230.

[156] M. Murate, M. Abe, K. Kasahara, K. Iwabuchi, M. Umeda, T. Kobayashi, *J. Cell Sci.* **2015**, *128*, 1627-1638.

[157] A. Makino, T. Baba, K. Fujimoto, K. Iwamoto, Y. Yano, N. Terada, S. Ohno, S. B. Sato, A. Ohta, M. Umeda, K. Matsuzaki, T. Kobayashi, *J. Biol. Chem.* **2003**, *278*, 3204-3209.

[158] K. Emoto, O. Kuge, M. Nishijima, M. Umeda, *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 12400-12405.

[159] U. Kato, K. Emoto, C. Fredriksson, H. Nakamura, A. Ohta, T. Kobayashi, K. Murakami-Murofushi, T. Kobayashi, M. Umeda, *J. Biol. Chem.* **2002**, *277*, 37855-37862.

[160] M. Zhao, *Amino Acids* **2011**, *41*, 1071-1079.

[161] M. Zhao, Z. Li, S. Bugenhagen, *J. Nucl. Med.* **2008**, *49*, 1345-1352.

[162] D. J. Craik, N. L. Daly, T. Bond, C. Waine, *J. Mol. Biol.* **1999**, *294*, 1327-1336.

[163] D. J. Craik, M. Cemazar, C. K. Wang, N. L. Daly, *Biopolymers* **2006**, *84*, 250-266.

[164] S. Troeira Henriques, D. J. Craik, *Biochemistry* **2017**, *56*, 669-682.

[165] P. W. Ford, K. R. Gustafson, T. C. McKee, N. Shigematsu, L. K. Maurizi, L. K. Pannell, D. E. Williams, E. Dilip de Silva, P. Lassota, T. M. Allen, R. Van Soest, R. J. Andersen, M. R. Boyd, *J. Am. Chem. Soc.* **1999**, *121*, 5899-5909.

[166] N. Oku, R. Krishnamoorthy, A. G. Benson, R. L. Ferguson, M. A. Lipton, L. R. Phillips, K. R. Gustafson, J. B. McMahon, *J. Org. Chem.* **2005**, *70*, 6842-6847.

[167] K. Makino, E. Nagata, Y. Hamada, *Tetrahedron Lett.* **2005**, *46*, 6827-6830.

[168] W. Xie, D. Ding, W. Zi, G. Li, D. Ma, *Angew. Chem. Int. Ed.* **2008**, *47*, 2844-2848.

[169] C. D. Andjelic, V. Planelles, L. R. Barrows, *Mar. Drugs* **2008**, *6*, 528-549.

[170] C. D. Cassilly, M. M. Maddox, P. T. Cherian, J. J. Bowling, M. T. Hamann, R. E. Lee, T. B. Reynolds, *PloS one* **2016**, *11*, e0154932.

[171] M. Ashburner, C. A. Ball, J. A. Blake, D. Botstein, H. Butler, J. M. Cherry, A. P. Davis, K. Dolinski, S. S. Dwight, J. T. Eppig, M. A. Harris, D. P. Hill, L. Issel-Tarver,

A. Kasarskis, S. Lewis, J. C. Matese, J. E. Richardson, M. Ringwald, G. M. Rubin, G. Sherlock, *Nat. Genet.* **2000**, *25*, 25-29.

[172] A. H. Tong, G. Lesage, G. D. Bader, H. Ding, H. Xu, X. Xin, J. Young, G. F. Berriz, R. L. Brost, M. Chang, Y. Chen, X. Cheng, G. Chua, H. Friesen, D. S. Goldberg, J. Haynes, C. Humphries, G. He, S. Hussein, L. Ke, N. Krogan, Z. Li, J. N. Levinson, H. Lu, P. Menard, C. Munyana, A. B. Parsons, O. Ryan, R. Tonikian, T. Roberts, A. M. Sdicu, J. Shapiro, B. Sheikh, B. Suter, S. L. Wong, L. V. Zhang, H. Zhu, C. G. Burd, S. Munro, C. Sander, J. Rine, J. Greenblatt, M. Peter, A. Bretscher, G. Bell, F. P. Roth, G. W. Brown, B. Andrews, H. Bussey, C. Boone, *Science* **2004**, *303*, 808-813.

[173] K. D. Atkinson, B. Jensen, A. I. Kolat, E. M. Storm, S. A. Henry, S. Fogel, *J. Bacteriol.* **1980**, *141*, 558-564.

[174] K. Atkinson, S. Fogel, S. A. Henry, *J. Biol. Chem.* **1980**, *255*, 6653-6661.

[175] K. Kiyono, K. Miura, Y. Kushima, T. Hikiji, M. Fukushima, I. Shibuya, A. Ohta, *J. Biochem.* **1987**, *102*, 1089-1100.

[176] P. Natarajan, J. Wang, Z. Hua, T. R. Graham, *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 10614-10619.

[177] T. Pomorski, R. Lombardi, H. Riezman, P. F. Devaux, G. van Meer, J. C. Holthuis, *Mol. Biol. Cell* **2003**, *14*, 1240-1254.

[178] M. Takar, Y. Wu, T. R. Graham, *J. Biol. Chem.* **2016**, *291*, 15727-15739.

[179] S. Chen, J. Wang, B. P. Muthusamy, K. Liu, S. Zare, R. J. Andersen, T. R. Graham, *Traffic* **2006**, *7*, 1503-1517.

[180] T. Mioka, K. Fujimura-Kamada, N. Mizugaki, T. Kishimoto, T. Sano, H. Nunome, D. E. Williams, R. J. Andersen, K. Tanaka, *Mol. Biol. Cell* **2018**, *29*, 1203-1218.

[181] M. A. Curto, M. R. Sharifmoghadam, E. Calpena, N. De Leon, M. Hoya, C. Doncel, J. Leatherwood, M. H. Valdivieso, *Genetics* **2014**, *196*, 1059-1076.

[182] Y. L. Chen, A. E. Montedonico, S. Kauffman, J. R. Dunlap, F. M. Menn, T. B. Reynolds, *Mol. Microbiol.* **2010**, *75*, 1112-1132.

[183] S. Matsunaga, N. Fusetani, K. Hashimoto, M. Walchli, *J. Am. Chem. Soc.* **1989**, *111*, 2582-2588.

[184] S. Matsunaga, N. Fusetani, *J. Org. Chem.* **1995**, *60*, 1177-1181.

[185] D. T. Youssef, L. A. Shaala, G. A. Mohamed, J. M. Badr, F. H. Bamanie, S. R. Ibrahim, *Mar. Drugs* **2014**, *12*, 1911-1923.

[186] K. Fukuhara, K. Takada, R. Watanabe, T. Suzuki, S. Okada, S. Matsunaga, *J. Nat. Prod.* **2018**, *81*, 2595-2599.

[187] C. A. Bewley, D. J. Faulkner, *J. Org. Chem.* **1994**, *59*, 4849-4852.

[188] E. W. Schmidt, C. A. Bewley, D. J. Faulkner, *J. Org. Chem.* **1998**, *63*, 1254-1258.

[189] C. A. Bewley, N. D. Holland, D. J. Faulkner, *Experientia* **1996**, *52*, 716-722.

[190] E. W. Schmidt, A. Y. Obraztsova, S. K. Davidson, D. J. Faulkner, M. G. Haygood, *Mar. Biol.* **2000**, *136*, 969-977.

[191] T. Mori, J. K. B. Cahn, M. C. Wilson, R. A. Meoded, V. Wiebach, A. F. C. Martinez, E. J. N. Helfrich, A. Albersmeier, D. Wibberg, S. Datwyler, R. Keren, A. Lavy, C. Ruckert, M. Ilan, J. Kalinowski, S. Matsunaga, H. Takeyama, J. Piel, *Proc. Natl. Acad. Sci. U.S.A.* **2018**, *115*, 1718-1723.

[192] S. Wada, S. Matsunaga, N. Fusetani, S. Watabe, *Mar. Biotechnol. (NY)* **1999**, *1*, 337-341.

[193] S. Wada, S. Matsunaga, N. Fusetani, S. Watabe, *Mar. Biotechnol. (NY)* **2000**, *2*, 285-292.

[194] S. Wada, S. Kantha, T. Yamashita, S. Matsunaga, N. Fusetani, S. Watabe, *Mar. Biotechnol. (NY)* **2002**, *4*, 571-582.

[195] A. Matsuyama, R. Arai, Y. Yashiroda, A. Shirai, A. Kamata, S. Sekido, Y. Kobayashi, A. Hashimoto, M. Hamamoto, Y. Hiraoka, S. Horinouchi, M. Yoshida, *Nat. Biotechnol.* **2006**, *24*, 841-847.

[196] A. Shirai, A. Matsuyama, Y. Yashiroda, A. Hashimoto, Y. Kawamura, R. Arai, Y. Komatsu, S. Horinouchi, M. Yoshida, *J. Biol. Chem.* **2008**, *283*, 10745-10752.

[197] R. A. Espiritu, N. Matsumori, M. Murata, S. Nishimura, H. Kakeya, S. Matsunaga, M. Yoshida, *Biochemistry* **2013**, *52*, 2410-2418.

[198] K. Cornelio, R. A. Espiritu, S. Hanashima, Y. Todokoro, R. Malabed, M. Kinoshita, N. Matsumori, M. Murata, S. Nishimura, H. Kakeya, M. Yoshida, S. Matsunaga, *Biochim. Biophys. Acta Biomembr.* **2019**, *1861*, 228-235.

[199] R. A. Espiritu, K. Cornelio, M. Kinoshita, N. Matsumori, M. Murata, S. Nishimura, H. Kakeya, M. Yoshida, S. Matsunaga, *Biochim. Biophys. Acta* **2016**, *1858*, 1373-1379.

[200] K. Cornelio, R. A. Espiritu, Y. Todokoro, S. Hanashima, M. Kinoshita, N. Matsumori, M. Murata, S. Nishimura, H. Kakeya, M. Yoshida, S. Matsunaga, *Bioorg. Med. Chem.* **2016**, *24*, 5235-5242.

[201] Y. Arita, S. Nishimura, R. Ishitsuka, T. Kishimoto, J. Ikenouchi, K. Ishii, M. Umeda, S. Matsunaga, T. Kobayashi, M. Yoshida, *Chem. Biol.* **2015**, *22*, 604-610.

[202] S. Nishimura, M. Tokukura, J. Ochi, M. Yoshida, H. Kakeya, *Chem. Biol.* **2014**, *21*, 1690-1699.

[203] S. Nishimura, K. Ishii, K. Iwamoto, Y. Arita, S. Matsunaga, Y. Ohno-Iwashita, S. B. Sato, H. Kakeya, T. Kobayashi, M. Yoshida, *PloS one* **2013**, *8*, e83716.

[204] S. Takahashi, K. Homma, Y. Zhou, S. Nishimura, C. Duan, J. Chen, A. Ahmad, M. A. Cheatham, J. Zheng, *Sci. Rep.* **2016**, *6*, 21973.

[205] C. Giampietro, M. C. Lionetti, G. Costantini, F. Mutti, S. Zapperi, C. A. La Porta, *Sci. Rep.* **2017**, *7*, 43669.

[206] Q. Xiong, M. Lin, W. Huang, Y. Rikihisa, *mBio* **2019**, *10*, e02783-02718.

[207] J. R. Edgar, P. T. Manna, S. Nishimura, G. Banting, M. S. Robinson, *eLife* **2016**, *5*.

[208] C. L. Bender, F. Alarcon-Chaidez, D. C. Gross, *Microbiol. Mol. Biol. Rev.* **1999**, *63*, 266-292.

[209] T. C. Helmann, A. M. Deutschbauer, S. E. Lindow, *Proc. Natl. Acad. Sci. U.S.A.* **2019**, *116*, 18900-18910.

[210] M. Anselmi, T. Eliseo, L. Zanetti-Polzi, M. R. Fullone, V. Fogliano, A. Di Nola, M. Paci, I. Grgurina, *Biochim. Biophys. Acta* **2011**, *1808*, 2102-2110.

[211] O. S. Ostroumova, P. A. Gurnev, L. V. Schagina, S. M. Bezrukov, *FEBS Lett.* **2007**, *581*, 804-808.

[212] A. M. Feigin, J. Y. Takemoto, R. Wangspa, J. H. Teeter, J. G. Brand, *J. Membr. Biol.* **1996**, *149*, 41-47.

[213] A. M. Feigin, L. V. Schagina, J. Y. Takemoto, J. H. Teeter, J. G. Brand, *Biochim. Biophys. Acta* **1997**, *1324*, 102-110.

[214] S. S. Efimova, A. A. Zakharova, L. V. Schagina, O. S. Ostroumova, *Eur. Biophys. J.* **2016**, *45*, 91-98.

[215] M. L. Hutchison, M. A. Tester, D. C. Gross, *Mol. Plant Microbe. Interact.* **1995**, *8*, 610-620.

[216] N. Taguchi, Y. Takano, C. Julmanop, Y. Wang, S. Stock, J. Takemoto, T. Miyakawa, *Microbiology* **1994**, *140 (Pt 2)*, 353-359.

[217] M. M. Grilley, S. D. Stock, R. C. Dickson, R. L. Lester, J. Y. Takemoto, *J. Biol. Chem.* **1998**, *273*, 11062-11068.

[218] H. Hama, D. A. Young, J. A. Radding, D. Ma, J. Tang, S. D. Stock, J. Y. Takemoto, *FEBS Lett.* **2000**, *478*, 26-28.

[219] S. D. Stock, H. Hama, J. A. Radding, D. A. Young, J. Y. Takemoto, *Antimicrob. Agents Chemother.* **2000**, *44*, 1174-1180.

[220] M. Toume, M. Tani, *FEMS Microbiol. Lett.* **2014**, *358*, 64-71.

[221] M. Satake, M. Murata, T. Yasumoto, T. Fujita, H. Naoki, *J. Am. Chem. Soc.* **1991**, *113*, 9859-9861.

[222] G. K. Paul, N. Matsumori, M. Murata, K. Tachibana, *Tetrahedron Lett.* **1995**, *36*, 6279-6282.

[223] G. K. Paul, N. Matsumori, K. Konoki, M. Murata, K. Tachibana, *Mar. Biotechnol.* **1997**, *5*, 124-128.

[224] N. Morsy, S. Matsuoka, T. Houdai, N. Matsumori, S. Adachi, M. Murata, T. Iwashita,

T. Fujita, *Tetrahedron* **2005**, *61*, 8606-8610.

[225] R. Echigoya, L. Rhodes, Y. Oshima, M. Satake, *Harmful Algae* **2005**, *4*, 383-389.

[226] N. Morsy, T. Houdai, S. Matsuoka, N. Matsumori, S. Adachi, T. Oishi, M. Murata, T. Iwashita, T. Fujita, *Bioorg. Med. Chem.* **2006**, *14*, 6548-6554.

[227] Y. Meng, R. M. Van Wagoner, I. Misner, C. Tomas, J. L. Wright, *J. Nat. Prod.* **2010**, *73*, 409-415.

[228] G. Nuzzo, A. Cutignano, A. Sardo, A. Fontana, *J. Nat. Prod.* **2014**, *77*, 1524-1527.

[229] A. Cutignano, G. Nuzzo, A. Sardo, A. Fontana, *Mar. Drugs* **2017**, *15*, 157.

[230] M. Satake, K. Cornelio, S. Hanashima, R. Malabed, M. Murata, N. Matsumori, H. Zhang, F. Hayashi, S. Mori, J. S. Kim, C. H. Kim, J. S. Lee, *J. Nat. Prod.* **2017**, *80*, 2883-2888.

[231] K. A. Martinez, C. Lauritano, D. Druka, G. Romano, T. Grohmann, M. Jaspars, J. Martin, C. Diaz, B. Cautain, M. de la Cruz, A. Ianora, F. Reyes, *Mar. Drugs* **2019**, *17*, E385.

[232] G. K. Paul, N. Matsumori, K. Konoki, M. Sasaki, M. Murata, K. Tachibana, *Harmful and Toxic Algal Blooms Yasumoto, T., Oshima, Y., Fukuyo, Y. Eds.* **1996**, 503-506.

[233] S. J. Huang, C. M. Kuo, Y. C. Lin, Y. M. Chen, C. K. Lu, *Tetrahedron Lett.* **2009**, *50*, 2512-2515.

[234] X. C. Huang, D. Zhao, Y. W. Guo, H. M. Wu, L. P. Lin, Z. H. Wang, J. Ding, Y. S. Lin, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3117-3120.

[235] X. C. Huang, D. Zhao, Y. W. Guo, H. M. Wu, E. Trivellone, G. Cimino, *Tetrahedron Lett.* **2004**, *45*, 5501-5504.

[236] K. Washida, T. Koyama, K. Yamada, M. Kita, D. Uemura, *Tetrahedron Lett.* **2006**, *47*, 2521-2525.

[237] N. Hanif, O. Ohno, M. Kitamura, K. Yamada, D. Uemura, *J. Nat. Prod.* **2010**, *73*, 1318-1322.

[238] T. Kubota, A. Takahashi, M. Tsuda, J. Kobayashi, *Mar. Drugs* **2005**, *3*, 113-118.

[239] T. Inuzuka, K. Yamada, D. Uemura, *Tetrahedron Lett.* **2014**, *55*, 6319-6323.

[240] M. Murata, S. Matsuoka, N. Matsumori, G. K. Paul, K. Tachibana, *J. Am. Chem. Soc.* **1999**, *121*, 870-871.

[241] Y. Wakamiya, M. Ebine, M. Murayama, H. Omizu, N. Matsumori, M. Murata, T. Oishi, *Angew. Chem. Int. Ed.* **2018**, *57*, 6060-6064.

[242] N. Morsy, T. Houdai, K. Konoki, N. Matsumori, T. Oishi, M. Murata, *Bioorg. Med. Chem.* **2008**, *16*, 3084-3090.

[243] R. A. Espiritu, N. Matsumori, M. Tsuda, M. Murata, *Biochemistry* **2014**, *53*, 3287-3293.

[244] M. Iwamoto, A. Sumino, E. Shimada, M. Kinoshita, N. Matsumori, S. Oiki, *Sci. Rep.* **2017**, *7*, 10782.

[245] T. Houdai, S. Matsuoka, N. Morsy, N. Matsumori, M. Satake, M. Murata, *Tetrahedron* **2005**, *61*, 2795-2802.

[246] T. Houdai, N. Matsumori, M. Murata, *Org. Lett.* **2008**, *10*, 4191-4194.

[247] S. M. Simonsen, L. Sando, D. C. Ireland, M. L. Colgrave, R. Bharathi, U. Goransson, D. J. Craik, *Plant Cell* **2005**, *17*, 3176-3189.

[248] M. Luo, E. A. Fadeev, J. T. Groves, *Nat. Chem. Biol.* **2005**, *1*, 149-153.

[249] C. F. McQueen, J. T. Groves, *J. Biol. Inorg. Chem.* **2018**, *23*, 995-1007.

[250] T. Ozaki, R. Sugiyama, M. Shimomura, S. Nishimura, S. Asamizu, Y. Katsuyama, H. Kakeya, H. Onaka, *Org. Biomol. Chem.* **2019**, *17*, 2370-2378.

[251] R. Sugiyama, T. Nakatani, S. Nishimura, K. Takenaka, T. Ozaki, S. Asamizu, H. Onaka, H. Kakeya, *Angew. Chem. Int. Ed.* **2019**, *58*, 13486-13491.