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REVIEWS

Mechanisms of Lipid-Mediated Regulation of the Pore-Forming Activity of Antimicrobial Agents: Studies on Planar Lipid Bilayers

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Abstract—Planar lipid bilayers are unique tools designed for modeling cell membranes and electrophysiological studies of ion channels embedded in them. Such model systems were invented to intentionally limit the complexity and multicomponent nature of cell membranes in order to analyze in detail the processes occurring there under well-controlled experimental conditions. Planar lipid bilayers make it possible to record single conduction events with a measured current of the order of a tenth of a picoampere. The relative simplicity of the method, the possibility of observing single molecular events and the high reproducibility of the results determine the unprecedented effectiveness of using planar lipid bilayers to identify key physical and chemical factors responsible for the regulation of the functioning of ion channels. This review is a collection of published data on the mechanisms of regulation of ion channels associated with the lipid microenvironment formed by various antimicrobial agents. The analysis allows us to consider lipids as molecular chaperones that ensure the formation of pores in targeted membranes by antimicrobial agents.

Keywords: planar lipid bilayers, ion channels, antimicrobial peptides and lipopeptides, polyene macrolide antibiotics

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INTRODUCTION

The invention of model lipid membranes has become an important stage in the formation of ideas about the structure and functioning of cell membranes, as well as in the development of an understanding of the mechanisms of antimicrobial and toxic effects of many substances. Currently, planar lipid bilayers are one of the most common and convenient systems for modeling cell membranes [1, 2]. Using this model, the effects of physico-chemical properties of the lipid matrix, the transmembrane distribution of electric potential and various chemicals on the ion transport through membranes is studied.

METHODS OF FORMATION OF PLANAR LIPID MEMBRANES

The first information about methods for the formation of artificial membranes dates back to the early 1960s, however, the development of a method for the formation of planar lipid membranes by Muller and Rudin [3, 4] should be considered as the starting point. The method is quite simple and consists in applying a drop of a lipid solution in an organic solvent with a pipette or brush to a hole in a Teflon partition that separates two compartments with an aqueous solution (Fig. 1a). Under the action of surface tension forces, the formed lipid film thins spontaneously to a thickness corresponding to two lipid molecules located in opposite monolayers. One of the significant disadvantages of such a model is the presence of solvent microlenses between lipid monolayers and a large torus of lipid solution at the boundary of the membrane attachment to the Teflon partition. There are several modifications of the Muller and Rudin method involving the use of various organic solvents with a low probability of microlens formation [6], removal of solvent lenses by bilayer freezing [7] or modification of an experimental chamber in order to obtain solvent-free lipid bilayers [8]; however, these options have not been widely used.

In 1972, Montal and Muller [9] proposed a fundamentally different method for the formation of planar lipid bilayers. The bilayers obtained in this way are "dry", that is, they do not include solvent lenses, but the technique itself is more laborious. At the beginning of the experiment, the hole in the Teflon partition separating the experimental chamber is treated with hexadecane or squalene; a lipid solution in hexane or pentane is applied to the surface of the solution in both compartments of the chamber, and then an artificial membrane is formed by bringing together pre-formed condensed lipid monolayers at the air-water interface on the hole in the Teflon film (Fig. 1b). As a result, the hydrocarbon chains of lipids in opposite monolayers turn out to face each other, and the hydrophilic heads

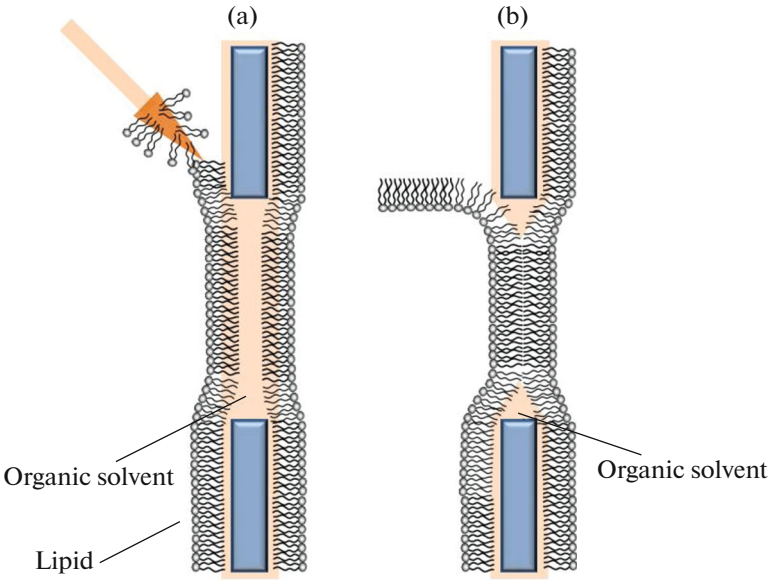

Fig. 1. Schematic representation of the formation of planar lipid membranes using the Muller–Rudin (a) and Montal–Muller (b) method [5].

of lipids are exposed to an aqueous solution. The advantages of the described method, in addition to the already mentioned absence of solvent microlenses between monolayers, include the possibility of creating asymmetric artificial membranes with different chemical composition of monolayers. The latter circumstance makes such models even more similar to cell membranes.

Subsequently, several other alternative methods for the formation of lipid bilayers for electrophysiological studies were developed, in particular, by combining lipid monolayers formed on the surface of two water droplets in hexadecane solution [10, 11], or using a pipette for "patch-clamp" [12].

Artificial lipid membranes obtained by such methods have proved to be extremely effective for electrophysiological measurements of ion channels, since they allow varying the chemical composition of membranes and their surrounding solutions over a wide range, as well as controlling a number of other important parameters with high accuracy, including the transmembrane potential difference. For this reason, the progress made in studying the processes of ion channel functioning is largely associated with the use of methods for the formation of planar lipid bilayers. The purpose of this review was to analyze the literature data on the mechanisms of regulation of ion channels formed by various antimicrobial agents associated with the lipid microenvironment.

PORE-FORMING ANTIMICROBIAL PEPTIDES

Antimicrobial peptides are generally considered to be relatively short peptides produced by the innate defense system of multicellular organisms to fight infectious pathogens, in particular, bacteria and fungi. Unicellular organisms are also able to synthesize compounds with pronounced antimicrobial effects, many of which have found their use as antibiotics. Almost all natural antimicrobial compounds are characterized by membrane activity, and a number of agents exert cytotoxic effect due to the formation of ion-permeable pores or channels in cell membranes of targeted organisms. Most of the information about the ability of antimicrobial agents to form pores in the membranes of target cells was obtained using model lipid membranes, and in particular, planar lipid bilayers. The most illustrative examples are given below.

Natural *gramicidin* is a heterogeneous mixture of several peptides produced by the bacterium *Brevibacillus brevis*, that, depending on the strain, includes such components as gramicidins A, B, C_D, D, and S [13, 14]. Figure 2 shows the chemical structure of gramicidin A. Alternating *L*- and *D*-amino acids in the linear sequence of gramicidin A allow the molecule to form a spiral structure in which polar groups line the inner cavity, and nonpolar side radicals are outside [15–17]. Such a conformation causes the incorporation of peptide molecules into lipid bilayers and their formation of pathways for the transport of ion, channels [18–21]. The introduction of gramicidin A on both sides of the membrane promotes the formation of ion channels by dimerization of monomers from opposite lipid monolayers [22, 23]. Gramicidin channels have been characterized in sufficient detail and are considered a classic object for modeling ion transport through membranes [24, 25]. The first studies on the channel-forming properties of gramicidin A, and in particular, the already mentioned paper of Hladky and Haydon in

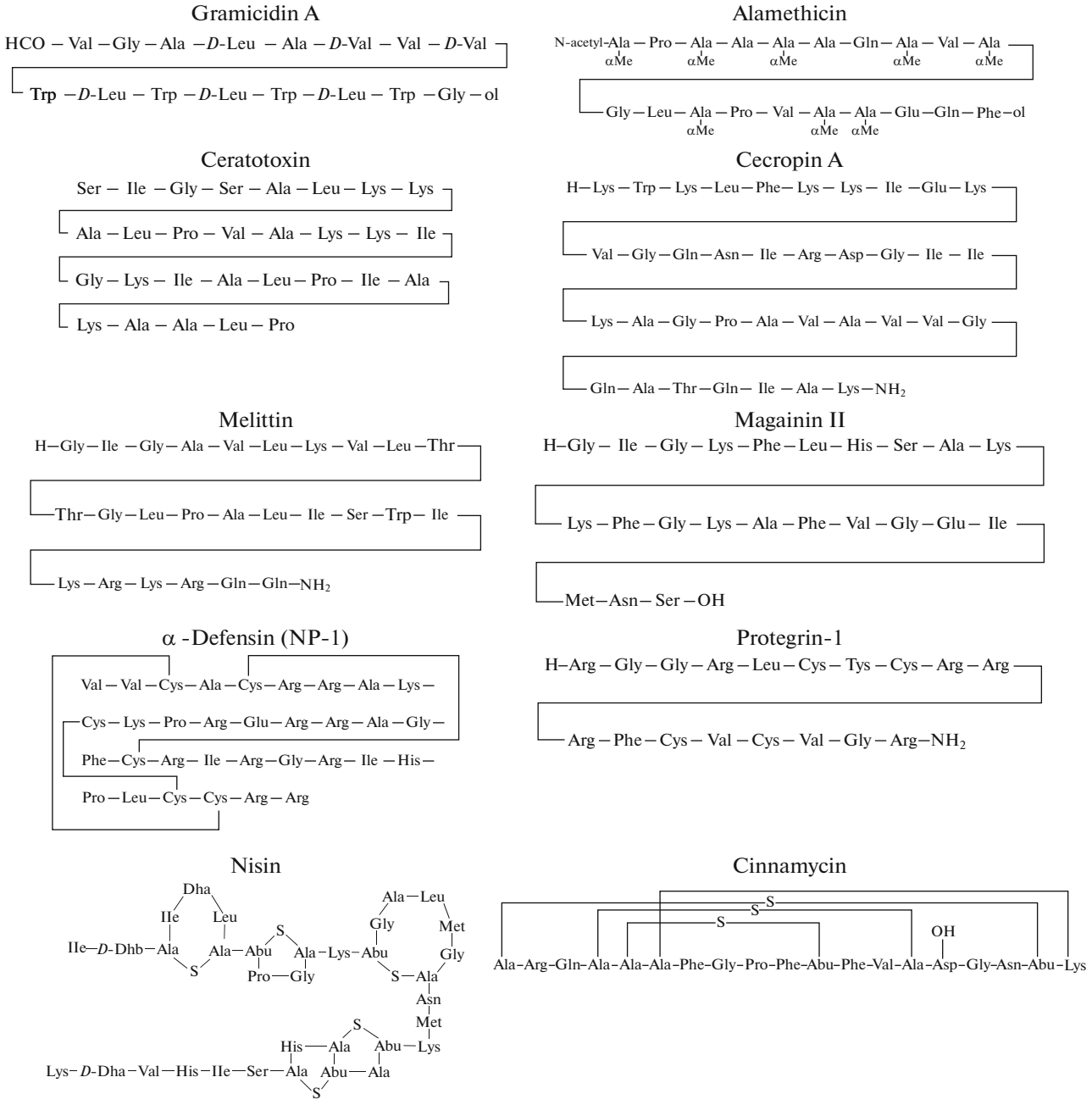

Fig. 2. Chemical structures of pore-forming antimicrobial peptides: gramicidin A, alamethicin, ceratotoxin, cecropin A, melittin, magainin II, α-defensin NP-1, protegrin-1, nisin and cinnamycin. Only *D*-enantiomers of amino acids are indicated. α-Me-Ala, α-methylalanyl; Phe-ol, phenylalaninol; Dha, dehydroalanine; Dhb, dehydrobutyrin; and Abu, α-aminobutyric acid.

the journal *Nature* [18], demonstrating recordings of gramicidin-induced step-like current fluctuations in a planar lipid bilayer of glycerol monooleate, are dated 1970. And despite 50 years of research, this object has not lost its relevance to this day.

Gramicidin channels are characterized by almost ideal cationic selectivity, they are permeable to monovalent cations [26–28] and are blocked by divalent cations and small iminium ions [29, 30]. The pore-forming activity of gramicidin A depends on the length of the hydrocarbon chains of membrane lipids, or rather on the thickness of the hydrophobic core [31, 32]. It was shown that the ion channel is stable if the length of the hydrophobic part of its transmembrane section corresponds to the thickness of the hydrocarbon backbone of the bilayer. In membranes, the thickness of the hydrophobic core of which exceeds the length of the hydrophobic part of the channel, the ion channel of the usual geometry is unstable and can transition from the state of two single helices associated "head to

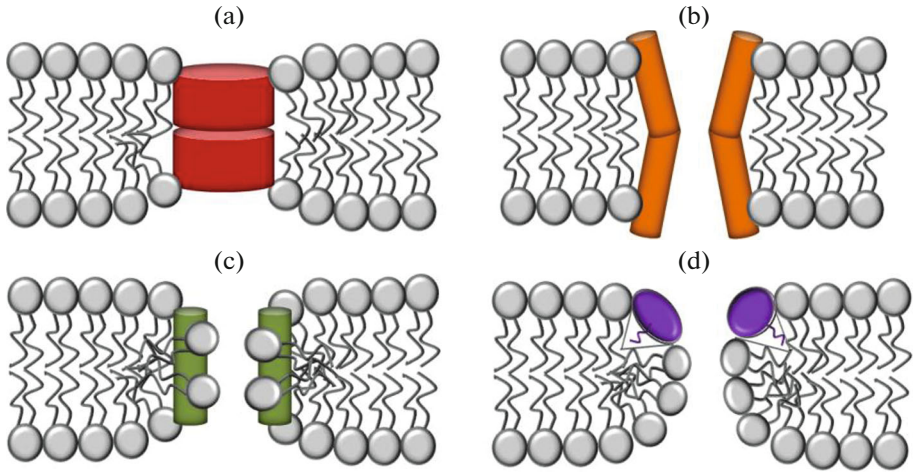

Fig. 3. Various models of ion channel formation by antimicrobial peptides: by dimerization of gramicidin A helices (a); association of alamethicin α-helices into a peptide "barrel" (b); formation of a peptide-lipid toroidal pore by melittin (c), and formation of an asymmetric lipopeptide-lipid pore by syringomycin E (d).

head" to the state of a more elongated double helix [33, 34]. The effect of many amphiphilic molecules on the conductance and lifetime of the gramicidin channel can be explained by their effect on the thickness of the hydrocarbon backbone of the membrane [19, 32]. The same hypothesis makes it possible to explain the peculiarities of the functioning of gramicidin channels in lipid bilayers undergoing phase separation [35]. In this case, lysolipids potentiate the pore-forming activity of gramicidin A by changing the energy of membrane deformation, which is associated with the molecular "shape" of membrane-forming lipids, and not with the thickness of the membrane [36]. The reverse effect was shown for the case of replacement of a membrane-forming lipid, lamellar phosphatidylcholine, with conical phosphatidylethanolamine [37]. Modification of the transmembrane distribution of lateral pressure also explained the effect of tubulin on the lifetime of gramicidin channels [38]. Based on the data on the effect of the elastic properties of the membrane on the activity of gramicidin A, a model of the channel formed by the peptide was proposed, which implies the presence of lipid mouths characterized by some positive spontaneous curvature (Fig. 3a) [39]. It should be noted that polar interactions between peptide molecules and the heads of membrane lipids can dominate the effect of hydrophobic mismatch in the regulation of the pore-forming function of gramicidin A [40]. It was found that gramicidin channels were also sensitive to an electric potential jump at the membrane-aqueous solution interface, in particular, to its unshielded (dipole) component [32, 41–44]. It has been shown that gramicidins B and C were also capable of forming ion channels in planar lipid bilayers, moreover, a mixture of three gramicidins (A, B and C) formed hybrid channels [45]. Natural acylated forms of gramicidins A and C are also channel formers [46].

Another frequently used classical model for studying transmembrane transport is ion channels formed by *alamethicin*. This antimicrobial peptide belongs to the family of peptaibols (polypeptide antibiotics rich in α-aminoisobutyric acid) and is produced by the fungus *Trichoderma viride*. The structure of alamethicin is shown in Fig. 2. The assumption that alamethicin is capable of inducing ion channels in planar lipid bilayers was first suggested in the study of Muller and Rudin in 1968 [47]. It was later shown that in the lipid microenvironment, alamethicin adopts an α-helical conformation and forms potential-dependent ion channels of predominantly cationic selectivity and multilevel conductance [48]. Modification of the amino acid composition of the peptide made it possible to determine the key residues in the alamethicin molecule responsible for the manifestation of pore-forming properties by this peptide [49–52]. The potential dependence of the opening of alamethicin channels is due to the high dipole moment of the peptide helices [48, 53]. Moreover, the dipole moment of the peptide also determines the sensitivity of its pore-forming activity to the dipole potential of the membrane [54]. The conductance of the channels depends on the concentration of alamethicin, the lipid composition of the membrane and the pH of the bilayer bathing solution [52, 55, 56]. It was assumed that the alamethicin channel is arranged according to the "barrel" principle (Fig. 3b) [49, 57]. According to this model, the pore formed by the peptide oligomer increases in size when additional monomers are embedded. This hypothesis explains the multilevel conductance of alamethicin channels. In addition, it is believed that the conducting cluster of alamethicin molecules has the shape of an hourglass (Fig. 3b). Such a refinement of the model was required by the stabilization of states with higher conductance detected in the experiment

when non-lamellar lipids were included in the membrane, which increased the pressure in the hydrophobic region [37]. Probably, due to the discrepancy between the length of the hydrophobic section of the alamethicin molecule and the thickness of the hydrocarbon backbone of the bilayer, transmembrane incorporation of the peptide causes elastic deformation of the membrane, and aggregation of alamethicin molecules into a conductive cluster reduces peptide-lipid interactions (moreover, the larger the cluster, the weaker the peptide-lipid interaction). Data on the effect of compounds modifying the spontaneous curvature of lipid monolayers on the pore-forming activity of alamethicin are in good agreement with this assumption [58].

It should be noted that the peptaibol family includes more than 50 peptides, and pore-forming activity was shown for a number of peptides. In particular, *zervamicins*, *trichorzines*, and *hypelcins* produced by *Emericellopsis satmosynnemata*, *Trichoderma harzianum*, and *Hypocrea peltata*, respectively, are also capable of forming voltage-dependent multilevel ion channels similar to alamethicins [59–62]. The hourglass-shaped peptide "barrel" model proposed for alamethicin was used to describe the structure of zervamicin channels (Fig. 3b) [63]. The peptaiboils *harzianin* and *saturnisporin* from *Trichoderma saturnisporum* and *T. harzianum*, respectively, form conducting oligomers of a smaller size than alamethicin in lipid bilayers including non-lamellar lipids [64]. The pore-forming properties of *trichotoxin* A50E from *T. viride* and *antiamoebin* isolated from strains of the fungi *Emericellopsis* and *Cephalosporium* were shown; these peptaibols form ion channels with predominantly one sublevel of conductance; they are organized by the hexamer and tetramer of the corresponding peptide, while the composition of the alamethicin channels may include 8–10 peptide molecules [65–67].

Channels formed by antimicrobial peptides of insects have similar characteristics to alamethicin. *Ceratotoxin*, an alpha-helical cationic peptide found in the secretory fluid of the accessory glands of the fruit fly *Ceratitis capitata*, exhibits pronounced antibacterial activity, which correlates well with the ability of the peptide to form ion channels [68]. Figure 2 shows the chemical structure of ceratotoxin A. In particular, in full accordance with the previously proposed model (Fig. 3b) this peptide forms potential-dependent multilevel channels in bilayers of lipids that increase pressure in the fatty acid tail region [68, 69]. Another example is *cecropins*, positively charged antibacterial peptides from the hemolymph of the butterfly *Hyalophora cecropia*. The key role in their antibacterial effect is played by electrostatic interactions with the negatively charged envelope of bacteria. Cecropins A, B, and D form potential-dependent ion channels in negatively charged planar lipid bilayers [70]. The structure of cecropin A is shown in Fig. 2. It was shown that, like the alamethicin and ceratotoxin channels, the pores formed by cecropins A and B are characterized by multilevel conductance [71]. At the same time, it turned out that, unlike alamethicin, the presence of non-lamellar lipids in the membrane composition, which create excessive pressure in the hydrocarbon core, is not a sufficient condition for the formation of channels by cecropins, and the applicability of the "barrel" model for cecropin channels should be evaluated in further studies. Using membrane-active compounds, it was also revealed that one of the factors regulating cecropin-induced macroscopic current is the membrane dipole potential, which may be due to the high dipole moment of the C-terminal domains of the peptide inserting into the membrane [71].

Melittin is an antimicrobial cationic linear polypeptide isolated from bee venom. The structure of this peptide is shown in Fig. 2. In the lipid microenvironment, the peptide adopts the conformation of the α-helix and causes a potential-dependent increase in the conductance of planar lipid bilayers by forming conductive tetramers [72]. The potential dependence of the pores formed by melittin is due to charge-dipole and dipole-dipole interactions between peptide molecules and the lipid bilayer. It is believed that, together with peptide helixes, the surface of the aqueous pore of the melittin channel is lined with several lipid heads, forming a peptide-lipid pore of toroidal geometry (Fig. 3c) [73–75]. In order to enhance the antimicrobial action of the peptide, its hybrids with cecropin were synthesized, which, like the parent molecules, exhibit pore-forming ability in planar lipid membranes [76, 77].

Magainins (I and II) are cationic peptides extracted from the skin of the spur frog *Xenopus laevis*. Figure 2 shows the chemical structure of magainin II. These peptides have a pronounced antimicrobial effect, causing lysis of bacterial and fungal cells. They exhibit cytolytic activity by forming aggregates from α-helical peptide pores in membranes including anionic lipids [78, 79]. The data concerning the cation-anion specificity of magainin channels are very contradictory. Reports can be found in the literature on both predominantly anionic and cationic functions [78, 80]. The parameter characterizing the cooperativity of the pore-forming action of magainin II, determined from the sigmoidal dependence of the peptide-induced current on its concentration, is 1.6 [80]. In this case, the elementary unit of conductance, the size of which is estimated by the parameter of cooperativeness, can be represented not only by the magainin monomer, but also by a dimer or even a peptide aggregate of a larger order. When studying the lipid specificity of the membrane action of magainin II, it was found that its channel-forming activity depends on the type of negatively charged lipid and the presence of sterol in the bilayer [79]. Many researchers agree that, like melittin, magainins form peptide-lipid toroidal pores (Fig. 3c) [75, 81–83]. This hypothesis explains the increase in the pore-forming activity of melittin and magainin

with an increase in positive spontaneous curvature using membrane modifiers and a decrease when nonlamellar lipids are included in the membrane composition, increasing pressure in the hydrophobic region of the bilayer [58, 84]. However, it is believed that the method of formation of pores by magainin (according to the principle of a peptide "barrel" or a peptide-lipid toroidal pore) depends on the lipid composition of the membrane. In particular, the pores induced by magainin I in negatively charged membranes can be successfully described in the framework of the first model [85].

Despite the fact that the primary sequence of *dermaseptin* and *pleurocidin*, alpha-helical cationic antimicrobial peptides isolated from the skin of the South American frog *Phyllomedusa bicolor* and winter flounder *Pseudopleuronectes americanus*, respectively, is homologous to ceratoxin, the peptide "barrel" model is not applicable to describe their pore-forming activity, and, most likely, these peptides form pores by a toroidal mechanism (Fig. 3c) [86, 87].

Antimicrobial peptides of mammals have a wide spectrum of action, extending to both Gram-positive and Gram-negative bacteria, as well as to some pathogenic fungi and even viruses, while many representatives also exhibit hemolytic activity. Among the antimicrobial peptides of mammals, *defensins*, *protegrins,* and *cathelicidins* should be mentioned. Figure 2 shows the structure of rabbit α-defensin NP-1 and protegrin-1 isolated from pig leukocytes. The ability to induce channel-like currents in planar lipid bilayers has been demonstrated for all these groups [88–90]. In artificial membranes containing negatively charged phospholipids, defensins form potential-dependent weakly anion-selective pores by association of β-sheets into oligomers [88, 89]. Like defensins, protegrins form ion channels due to the oligomerization of β-sheets [91]. The probability of formation of channels by protegrins also depends on the lipid composition of the membrane. These peptides are characterized by higher specificity for membranes containing negatively charged lipids (including bacterial lipopolysaccharides) and nonlamellar lipids [91, 92]. Protegrins apparently induce transmembrane channels corresponding to the toroidal model, in which aqueous pores with positive spontaneous curvature are lined with both peptides and heads of membrane lipids (Fig. 3c) [75]. It has been shown that in negatively charged lipid membranes, regardless of the sign of the transmembrane voltage, cathelicidin tritrpticin forms cation-selective channels, for the description of the properties of which the toroidal model is also applicable (Fig. 3c) [90].

The cationic polycyclic peptide *nisin* synthesized by *Streptococcus lactis* bacteria belongs to the group of lantibiotics and has a wide range of antimicrobial action, including many Gram-positive bacteria. Its molecule contains residues of three non-standard amino acids, dehydroalanine (Dha), dehydrobutyrin (Dhb) and aminobutyric acid (Abu), as well as five rings formed by one lanthionine and four β-methyllanthionine bridges (Fig. 2) [93]. It is believed that as a result of the interaction of the lantibiotic with lipid II, a molecule involved in the translocation of peptidoglycan blocks through the bacterial membrane [94], pores are formed in it and, as a result, the vital activity of the cell is violated [95–98]. Using planar lipid membranes, it was shown that the presence of lipid II in the membrane is not a prerequisite for the manifestation of nisin's ability to induce ion channels, the presence of negatively charged phospholipids is sufficient, and the lantibiotic shows the greatest affinity for cardiolipin-containing membranes [99–101].

The lanthibiotics *cinnamycin* and *duramycin* produced by *Streptomyces* sp. demonstrate pore-forming activity in lipid bilayers, including lipids that increase pressure in the region of the hydrophobic core [102]. The structure of cinnamycin is shown in Fig. 2. It should be noted that the elucidation of the mechanisms underlying the pore-forming ability of lantibiotics requires more detailed studies, including using artificial lipid membranes.

PORE-FORMING LIPOPEPTIDES

A number of microorganisms synthesize bioactive molecules belonging to the class of cyclic lipopeptides. The simultaneous presence of a ring-enclosed peptide "head", which includes charged and/or polar amino acid residues, and a hydrophobic hydrocarbon "tail" makes lipopeptide molecules amphiphilic, and therefore ensures their high affinity for membranes. It has been found that a number of membrane-active cyclic lipopeptides are capable of forming ion-permeable pores in lipid bilayers. The chemical structures of some compounds are shown in Fig. 4.

Bacilli produce cyclic lipopeptides belonging to three different groups, *iturins*, *surfactins* and *fengycins* [103]. *Iturin* A and C, *bacillomycins* D, F, L and LC, and *mycosubtilin* are the main representatives of the iturin family and were found in *B. subtilis* and *B. amyloliquefaciens* [104, 105]. Surfactin or its closely related variants, such as lichenizin, esperin and pumilacidin, were isolated from *B. coagulans*, *B. pumilus* and *B. licheniformis*, respectively [106], and the group of fengycins synthesized by *B. subtilis*, *B. cereus* and *B. thuringiensis* includes fengycins A and B [107, 108]. It was found that iturin A, mycosubtilin, bacillomycins L and D, surfactin and fengycin, characterized by pronounced antifungal activity, form ion channels in model lipid membranes [109–114]. However, it should be noted that a detailed characterization of the ion channels formed by lipopeptides has not yet been carried out, and only fragmentary information can be found in the literature. It is believed that the process of pore formation implies self-association of lipopeptide molecules, but the cooperative action was shown only for surfactin and fengycin [112, 114]. It was revealed

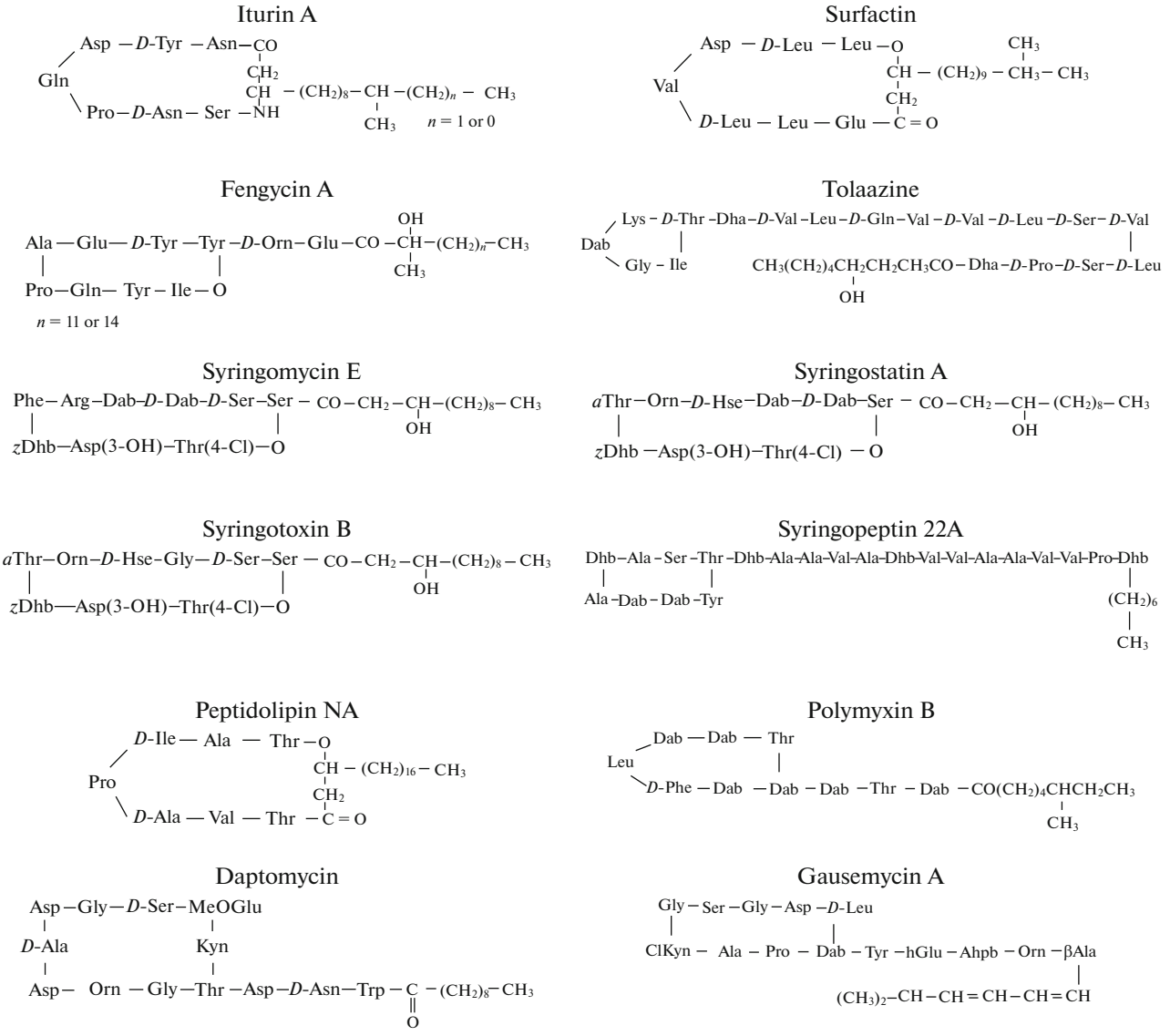

Fig. 4. Chemical structures of pore-forming antimicrobial cyclic lipopeptides: iturin A, surfactin, fengycin A, tolaazine, syringomycin E, syringostatin A, syringotoxin B, syringopeptin 22A, peptidolipin NA, polymyxin B, daptomycin and gausemycin A. Only *D*-enantiomers of amino acids are indicated. Orn, ornithine; Dab, 2,4-diaminobutyric acid; Dhb, 2,3-dehydroaminobutyric acid; Thr(4-Cl), 4-chlortreonine; MeOGlu, 3-methylglutamic acid; Kyn, kynurenine; Ahpb, 2-amino-4-hydroxy-4-phenylbutyric acid.

that iturin channels are characterized by a weak anionic conductance, while surfactin and fengycin channels are characterized mainly by a cationic conductance [110, 112]. It was found that the pore-forming activity of surfactin depends on the dipole potential of the membrane [113], and the inclusion of cholesterol in the bilayer potentiates the pore-forming ability of iturin A and mycosubtilin [110, 111].

Pseudomonads also synthesize cyclic lipopeptides of various chemical structure, which, in addition to phytotoxic activity, exhibit antimicrobial activity. *Tolaazine* is an antimicrobial lipopeptide produced by *Pseudomonas tolaasii* and causes brown spotting of cultivated edible mushrooms. In lipid bilayers, tolaazine induces ion channels of different conductance, which are blocked by zinc ions [115]. *Pseudomonas syringae* epiphyte bacteria synthesize several types of phytotoxins of a lipopeptide nature, *syringomycins*, *syringostatins*, *syringotoxins* and *syringopeptins* [116]. From the point of view of the ability to form pores, the most characterized representative is syringomycin E. It has been shown that this lipopeptide induced stable potential-dependent ion channels of multilevel conductance with predominantly anionic selectivity [117–119]. It has been found that syringomycin channels of higher conductance are clusters of several elementary conduction subunits with a common gate mechanism

[119, 120]. The dependence on the lipid composition of the membrane and the difference in the size of the pore mouths indicate that syringomycin channels are arranged according to the principle of a lipopeptide-lipid toroidal pore, the smaller mouth of which is formed by lipopeptide molecules, and the larger mouth by molecules of membrane lipids (Fig. 3d) [121, 122]. It was also found that the channel-forming activity of syringomycin E is influenced by the dipole potential of the membrane [123, 124], and the pores are blocked by local anesthetics of the aminoamide series [125]. Similarly to syringomycin E, ion channels are formed by syringostatin A, syringotoxin B, and syringopeptins 22A and 25A [126–130].

The ability to form ion-permeable pores in lipid bilayers has also been shown for the antifungal lipopeptide *peptidolipin NA* from *Nocardia asternides* [109].

The most studied antibacterial cyclic lipopeptides are *polymyxins* B and E (also known as colistin), obtained from the bacterium *Paenibacillus polymyxa*. Polymyxins were discovered in 1974 and are currently used as antibiotics of last resort for the treatment of infections caused by polyresistant Gram-negative bacteria, including *Pseudomonas aeruginosa*. It is believed that the bactericidal effect of polymyxins is due to their binding to lipopolysaccharides of Gram-negative bacteria and the formation of ion-permeable pores in bacterial membranes [131–133]. The high toxicity of these antibiotics should be noted, which is also associated with the ability to induce pores in the membranes of target cells [134]. The data obtained using planar lipid bilayers showed that polymyxin B predominantly interacts with negatively charged membranes, and, most likely, the dimer and hexamer of the lipopeptide are involved in the formation of pores in phospholipid and lipopolysaccharide-enriched bilayers, respectively [135, 136]. The pore-forming ability of polymyxin B significantly depends on the shape of membrane lipids, which indicates that the antibiotic forms toroidal lipopeptide-lipid pores (Fig. 3d) [136]. It has been shown that modifiers that reduce the dipole potential of the membrane potentiate the formation of pores by polymyxin B [136].

Malacidins belong to the family of negatively charged lipopeptide antibiotics that act on bacteria in a calcium-dependent manner. Daptomycin, the most famous representative of this family, is one of the drugs of last resort for the treatment of infections caused by Gram-positive bacteria. In 1985, this antibiotic was first isolated from *Streptomyces roseosporus*, and only in 2003 was approved by the FDA for use. Despite the fact that the pore-forming activity of daptomycin is widely discussed in the literature, most of the information was obtained during experiments on the permeability of liposomes to fluorescent dyes and potassium, and the real channel-like current fluctuations induced by this lipopeptide were demonstrated only in several studies using planar lipid bilayers. It has been found that in the presence of calcium, daptomycin forms potential-sensitive oligomeric pores of various conductance [137]. The smallest unit of conductance is probably the dimer. However, conducting oligomers of a larger order (tetramers and pentamers) function in the membrane more often. An increase in tolerance to the pore-forming action of daptomycin with an increase in the content of cardiolipin in model and cell membranes [138] may indicate that this cyclic lipopeptide forms toroidal lipopeptide-lipid pores (Fig. 3d).

It has recently been discovered that cyclic lipopeptides *gausemycins* A and B from streptomyces, showing promising activity against resistant strains of Gram-positive bacteria, also realize their antibacterial effect through the formation of ion-permeable pores, and their ability to form channels depends on the type of membrane-forming lipids and the presence of calcium ions [139, 140].

PORE-FORMING AGENTS OF NON-PEPTIDE NATURE

Polyene macrolide antibiotics are a large class of non-peptide compounds produced by actinomycetes. The chemical structure of some representatives is shown in Fig. 5. The molecules of these antibiotics are based on a macrolide ring containing a rigid hydrophobic polyene chain (usually consisting of several conjugated double bonds, the number of which is the main criterion for classifying compounds) and a hydrophilic chain rich in hydroxyl radicals. Polyene macrolides are the most effective antifungal drugs that have been used to treat systemic mycoses for several decades. The use of polyene antibiotics for therapeutic purposes is associated with an increased risk of developing a large number of serious side effects, such as nephropathy, anemia, thrombophlebitis and arrhythmia [141, 142].

All researchers agree that the antifungal effect of polyene antibiotics is determined by their membrane activity, and the key factor is the presence of sterols in the membranes of target cells. Thus, the presence of ergosterol in the membranes of pathogenic fungi determines the antifungal activity, and the presence of cholesterol in the membranes of mammalian cells determines the toxic effect of the drugs. Moreover, the affinity of polyene antibiotics for ergosterol is higher than for cholesterol, but the therapeutic window due to this difference is not so large, which determines the high toxicity of these drugs and the possibility of their use only as "last resort" antibiotics. The main differences in the views of researchers mainly relate to the molecular mechanisms of the membrane action of polyene macrolides. The dominant concept is based on a violation of the water-electrolyte balance during the formation of transmembrane pores by these agents [143], and an alternative hypothesis links the violation of the vital activity of target cells with the extraction of membrane sterols by polyenes [144, 145].

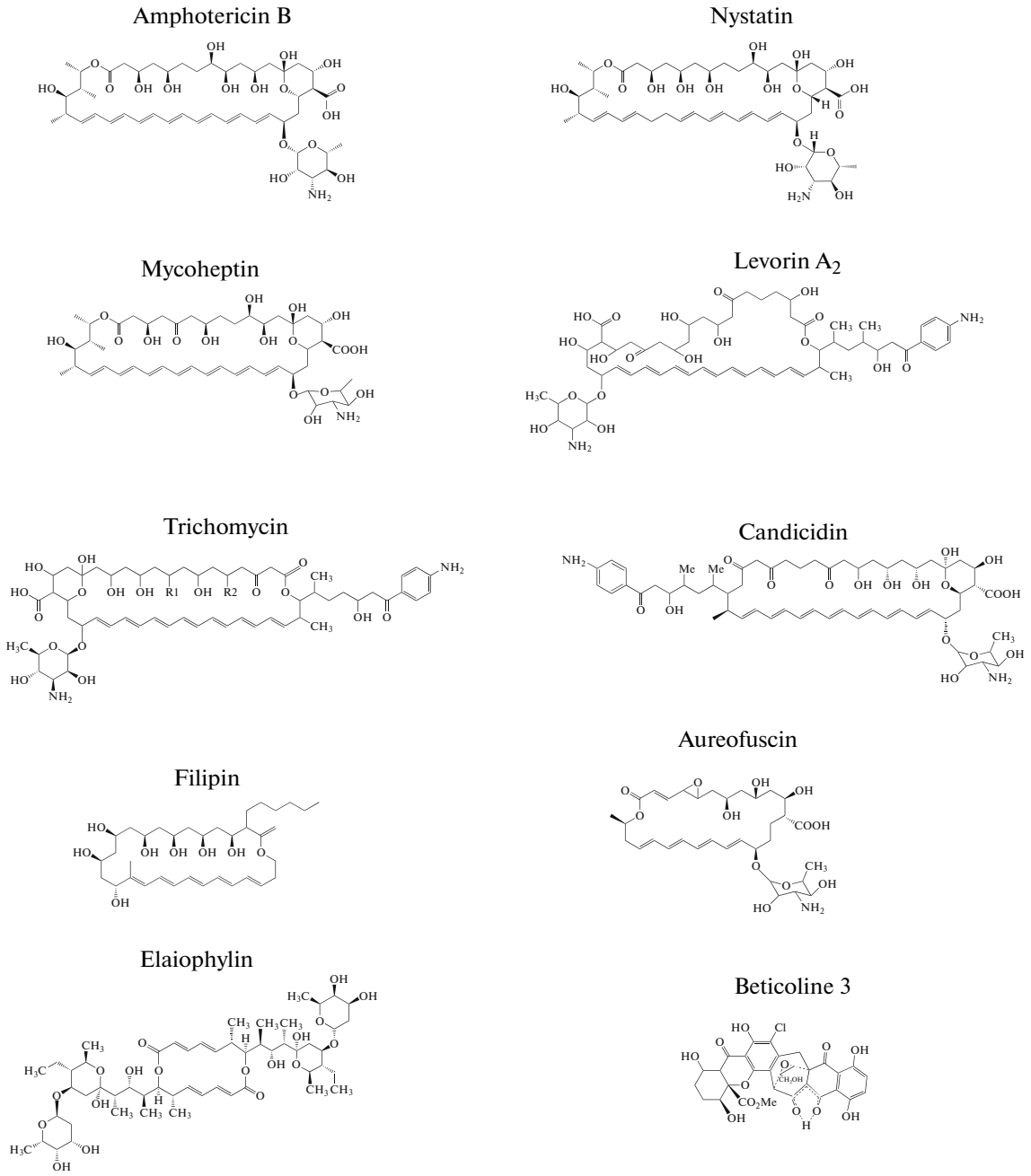

Fig. 5. Chemical structure of pore-forming antimicrobial agents of non-peptide nature: amphotericin B, nystatin, mycoheptin, levorin A_2, trichomycin, candicidin, filipin, aureofuscin, elaiophylin and beticolin 3.

Discrete channel-like fluctuations of current flowing through planar lipid bilayers treated with the most commonly used polyene antibiotics amphotericin B and nystatin in clinical practice were first demonstrated in the work of Ermishkin and colleagues in 1976 [146]. Subsequently, the ability to form pores was also revealed in mycoheptin, levorin A_2, trichomycin, candicidin, filipin, aureofuscin and elaiophylin [147–149].

The exact architecture of the pores formed by polyene antibiotics is still a subject of scientific debate. It is commonly believed that after adsorption on the membrane, polyene molecules bind to sterols, and then the polyene-sterol complexes (according to various estimates from 6 to 8) form a cylindrical "half-hole" according to the "barrel" principle due to electrostatic interactions between antibiotic molecules. In this case, the residues of mycosamines and the carboxyl groups of amphotericin B and nystatin oriented perpendicular to the membrane plane are turned into the aqueous phase, the hydroxyl groups of the hydrophilic

chain line the aqueous pore, and polyene fragments interact with sterol molecules [147]. In the case of antibiotic addition on only one side of the membrane, the half-pore forms an asymmetric channel with a lipid mouth on the opposite side of the bilayer [150–152]. To describe the structure of the asymmetric polyene channel, the model proposed for syringomycin E can be used (Fig. 3d). When amphotericin B or nystatin is introduced on both sides of the lipid bilayer, two half-pores in opposite lipid monolayers are associated by forming hydrogen bonds between hydroxyl groups, forming a symmetrical channel similar to the dimerization of gramicidin A (Fig. 3a) [143, 151, 153, 154]. The threshold concentrations of the antibiotic required for the observation of asymmetric and symmetric polyene channels, as well as their anion-cation specificity, are different [151, 154, 155]. The conductance of single anion-selective symmetric amphotericin B channels is a function of the dipole potential of the membrane [156, 157], and asymmetric cation-specific amphotericin B and nystatin channels, due to the presence of a lipid mouth with positive spontaneous curvature, are sensitive to changes in the elastic properties of the membrane when modifiers are introduced [158–160].

Beticolins are a group of non-peptide phytotoxins of perylene quinone nature produced by the fungus *Cercospora beticola*, which also exhibit antimicrobial activity. Figure 5 shows the chemical structure of beticolin 3. It was shown that beticolins form Mg^{2+}-dependent weakly selective ion channels with multiple levels of conductance in planar lipid bilayers [161, 162]. The multilevel conductance of beticolin channels is due to the cluster organization of elementary conducting subunits. There is no exact information in the literature about the structure of beticolin channels. It was suggested that transmembrane pores are formed by the association of several beticolin dimers into a conductive tubular structure [161].

CONCLUSIONS

The development of methods for creating artificial models of cell membranes has largely determined progress in the study of the mechanisms of antimicrobial action of exogenous compounds. The studies reviewed in this paper on the pore-forming activity of various antimicrobial compounds of natural origin indicate the key role of the lipid composition of target cell membranes. The presence of negatively charged lipids in bacterial membranes promotes the adsorption of, as a rule, positively charged antimicrobial peptides on the surface and ensures their functionally significant folding (in particular, the acquisition of an α-helical conformation). Other important factors are the thickness of the target membrane, which determines the difference between the thickness of the hydrocarbon core of the lipid bilayer and the length of the hydrophobic section of the pore-forming structure in the open state, as well as the presence of non-lamellar lipids, which can reduce the energy cost of membrane deformation in case of a mismatch. This gives reason to consider lipids of target cell membranes as molecular chaperones that ensure the process of pore formation by antimicrobial agents. In addition, due to the presence of a charge and/or a sufficiently high dipole moment in most antimicrobial compounds, their channel-forming activity can be regulated by the dipole potential of the membrane. Thus, membrane modifiers capable to significantly change the elastic properties of the lipid matrix and the dipole potential of the membrane can be agonists of the pore-forming activity of antimicrobial agents, that is, they can be used in combination therapy to increase the therapeutic index of a drug, which is extremely important both from the point of view of finding ways to solve the problem of high toxicity of pore-forming antibiotics and combating resistant strains of pathogenic microorganisms. Further research in this area, including using planar lipid bilayers, can contribute to a better understanding of the patterns underlying the formation of ion channels by exogenous compounds and elucidate the features of the molecular landscape characterizing these processes.

ABBREVIATIONS AND NOTATION

Dha	dehydroalanine
Dhb	dehydrobutyrin
Abu	aminobutyric acid

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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