Review

Lipid-Centric Approaches in Combating Infectious Diseases: Antibacterials, Antifungals and Antivirals with Lipid-Associated Mechanisms of Action

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Abstract: One of the global challenges of the 21st century is the increase in mortality from infectious diseases against the backdrop of the spread of antibiotic-resistant pathogenic microorganisms. In this regard, it is worth targeting antibacterials towards the membranes of pathogens that are quite conservative and not amenable to elimination. This review is an attempt to critically analyze the possibilities of targeting antimicrobial agents towards enzymes involved in pathogen lipid biosynthesis or towards bacterial, fungal, and viral lipid membranes, to increase the permeability via pore formation and to modulate the membranes’ properties in a manner that makes them incompatible with the pathogen’s life cycle. This review discusses the advantages and disadvantages of each approach in the search for highly effective but nontoxic antimicrobial agents. Examples of compounds with a proven molecular mechanism of action are presented, and the types of the most promising pharmacophores for further research and the improvement of the characteristics of antibiotics are discussed. The strategies that pathogens use for survival in terms of modulating the lipid composition and physical properties of the membrane, achieving a balance between resistance to antibiotics and the ability to facilitate all necessary transport and signaling processes, are also considered.

Keywords: membrane; lipid biosynthesis; antimicrobials; antibiotics; drug resistance; pore formation; ion channels; membrane curvature stress

1. Introduction

Here, we provide an overview of antibacterial, antifungal, and antiviral agents that target lipid biosynthesis and modulate the properties of pathogen membranes, including pore formation, the induction of curvature stress, and full disruption. Taking into account the emphasis on the lipid-associated mechanisms of action of potent antimicrobial drugs, to compare various agents that inhibit lipid biosynthesis, the concentrations causing a twofold decrease in the activity of appropriate enzymes are presented. The threshold concentration in the membrane bathing solution is chosen to quantitatively characterize the effectiveness of various pore-forming antibiotics. The modulation of the properties of the host cell plasma membranes, targeting enzymes engaged in the regulation of lipid metabolism and the biosynthesis of pathogens’ cell wall components, are not reviewed.

2. Antibacterials with Lipid-Associated Mechanisms of Action

We focus on two modes of targeting antibacterial agents towards pathogen membranes: (i) indirect action via inhibition of the biosynthesis of membrane lipids; (ii)
primary interaction with lipids, which results in the disruption of the functioning of bacterial membranes. These fundamentally different possibilities are considered below.

2.1. Inhibitors of Membrane Lipid Biosynthesis in Bacteria

Despite the fact that bacterial cell wall biosynthesis inhibitors, especially β-lactams and glycopeptide antibiotics, inhibiting the synthesis of the peptidoglycan layer, are the most effective and extensively used classes of antibiotics [1,2], they are not covered in this work, which focuses on targeting the lipid membrane. This part of the review concerns the key enzymes in the biosynthesis of membrane lipids in bacteria and their inhibitors. Possible ways to regulate the biosynthesis, transport, and degradation of lipids are not considered.

2.1.1. Biosynthesis of Fatty Acids of Bacterial Membrane Lipids

The search for selective inhibitors of enzymes participating in bacterial pathways for lipid biosynthesis is a good strategy to find novel antibiotics due to the fact that a certain lipid composition of the bacterial membrane is necessary for its proper functioning, and there is a difference in the principal organization of lipid biosynthesis in bacteria and mammals. In particular, the membrane's fatty acid composition is very important for metabolic plasticity and the growth rate of bacteria. Bacterial and mammalian fatty acid synthases (FAS) have various types. Bacterial and plant fatty acid synthases belong to type II (FASII), and each reaction is catalyzed by distinct single-functional small proteins. Type I fatty acid synthases (FASI), present in mammals and yeast, are composed of one polypeptide chain, and each stage of FAS is accomplished by a various functional domain of this multidomain protein. Figure 1 summarizes the information about the key enzymes of FASII in different bacteria. Due to another principal organization of mammalian FASI, the specific inhibitors of key enzymes of bacterial FASII are expected to be good candidates for the development of low-toxicity antibacterials. Molecules that effectively inhibit fatty acid synthesis in bacteria are discussed extensively in the text below. Table 1 provides information on some specific inhibitors of each known enzyme, as well as their inhibitory concentrations. A demonstration of the ability of the compound to inhibit the activity of the appropriate enzyme in in vitro tests can be considered as direct evidence in favor of a lipid synthesis-related mechanism of action and a specific molecular target. For this reason, the table presents only those inhibitors for which such information can be found in the available literature. The minimum inhibitory concentrations against various bacteria are not presented in Table 1 due to the high variability depending on the bacterial strain. Examples of the most common/known inhibitors are also shown in Figure 1 and marked with a black box. Next, we analyze the possibility of pharmacologically influencing this bacterial pathway and discuss more promising chemical scaffolds for further optimization. It should be taken into account that the therapeutic strategy, in addition to analyzing the inhibitory concentrations, must take into account the risks of developing resistance to the antibiotic and the side effects of its application.
Figure 1. Schematic representation of the synthesis of fatty acids of bacterial membrane lipids. The red, blue, and purple ellipses indicate that the enzyme is produced by Gram-positive, Gram-negative, or both Gram-positive and Gram-negative bacteria, respectively. Some examples of enzyme inhibitors are shown in the black box. Abbreviations: AccC—biotin carboxylase; AccB—biotin carboxyl carrier protein; AccAD—biotin carboxyl transferase; FabD—malonyl-CoA acyl carrier protein (ACP) transacylase; FabH, FabF, and FabB—β-ketoacyl-ACP synthase III (KAS III), II (KAS II), and I (KAS I), respectively; mtFabH—FabH homolog of Mycobacterium tuberculosis; FabG—NADPH-dependent β-ketoacyl-ACP reductase; FabZ—β-hydroxyacyl-ACP dehydratase; FabA/FabQ/FabN—bifunctional β-hydroxyacyl-ACP dehydratase/trans-2,3-decenoyl-ACP isomerase; FabM—trans-2,3-decenoyl-ACP isomerase; FabI/FabK/FabL/FabV—trans-2-enoyl-ACP reductase; PlsX—phosphate acyltransferase; PlsY—acyl-phosphate:glycerol-3-phosphate acyltransferase; PlsB—glycerol-3-phosphate acyltransferase; PlsC—1-acyl-sn-glycerol-3-phosphate acyltransferase; BCA—branched-chain fatty acids; LCFA—long-chain fatty acids; LPA—lysophosphatidic acid; PA—phosphatidic acid; UFA—unsaturated fatty acids; SFA—saturated fatty acids; CFA—cyclopropane fatty acids; R, R′, R″, and R‴—fatty acid hydrocarbon radicals.

Table 1. Major inhibitors of bacterial FASI.

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<th>Inhibitor</th>
<th>Structure</th>
<th>Enzyme</th>
<th>Origin</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;, μM</th>
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<td>(R)-2-[(2-chlorobenzylamino)-1- (2,3-dihydro-1H-inden-1-yl)-1 H-imidazo[4,5-b]pyridine-5-car boxamide]</td>
<td><img src="image2.png" alt="Structure" /></td>
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<td>[5]</td>
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<td>Organism</td>
<td>IC₅₀ (µM)</td>
<td>Reference</td>
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macrolactin S  

macrolactin B  

NAS-21  

NAS-91  

emodin  

mangostin  

stictic acid  

1,4-naphthoquinone  

juglone  

triclosan  

AFN-1252  

xanthorrhizol  

complestatin  

FabG  S. aureus  130  [17]  

FabG  S. aureus  100  [17]  

FabZ  M. smegmatis  360  [18]  

FabZ  M. smegmatis  498  [18]  

FabZ  F. tularensis  43.1 ± 9.2  [19]  

FabZ  Y. pestis  29.7 ± 6.0  [19]  

FabZ  H. pylori  9.70 ± 1.0  [20]  

FabZ  F. tularensis  7.7 ± 2.0  [19]  

FabZ  Y. pestis  6.1 ± 1.4  [19]  

FabZ  E. coli  20 ± 1  [22]  

FabZ  F. tularensis  5.4 ± 1.4  [19]  

FabZ  Y. pestis  5.3 ± 1.0  [19]  

FabZ  H. pylori  30 ± 4  [22]  

FabB  E. coli  0.98  [23]  

FabI  S. aureus  0.44–0.66  [23–25]  

FabI  P. falciparum  0.05–2  [15,26]  

FabI  C. trachomatis  0.32 ± 0.08  [27]  

FabI  E. coli  17.1 ± 1.8  [28]  

FabI  S. aureus  0.5  [25]  

FabK  S. pneumoniae  10  [25]
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<th>Organism</th>
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<td><em>H. pylori</em></td>
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<td>[35]</td>
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IC<sub>50</sub> is determined as concentration required for 50% inhibition of activity of appropriate enzyme.

The acetyl-CoA carboxylase is represented by a multiprotein complex, containing biotin carboxylase (AccC), the biotin carboxyl carrier protein (AccB), and biotin carboxyl transferase (AccAD), and performing the carboxylation of acetyl-CoA to generate...
malonyl-CoA (Figure 1) [36]. Via virtual screening and optimization of the small molecule library, antibacterials aminooxazoles and benzimidazoles were identified as potential AccC inhibitors (Table 1) [3,4]. Comparing the IC₅₀ values, the optimization of the benzimidazole scaffold seems to be a more promising way to find more potent AccC inhibitors, but the appropriate toxicity tests should be carried out to estimate the safe therapeutic window. The biotin carboxylation step is also known to be inhibited by pyrrololidone and equisetin [37]. The selectivity of pyrrololidone and equisetin between bacterial and human cells does not exceed 10 [37], and the mechanisms of their toxic action should be elucidated to increase the therapeutic window for more potent AccC inhibitors.

The broad-spectrum antibacterial activity of pyrrolidone derivatives, particularly moiramide B and andrimid, is referred to as targeting AccAD (Table 1) [5,38–40]. An in silico evaluation of andrimid showed no systemic toxicity [41]. However, bacterial resistance to andrimid arising from a single amino acid mutation in AccAD was found [42]. A promising approach, including the development of dual-use conjugated inhibitors of acetyl-CoA carboxylase composed of covalently linked motifs of aminooxazole (interacting with AccC) and moiramide B (targeting AccAD), was proposed to lower the frequency of strain resistance [43]. Preliminary studies on the antibacterial mechanism of yanglingmycin exhibited the potent inhibition of AccAD, which led to fatty acid and lipid biosynthesis being blocked, and, as a result, cell membrane destruction [44]. Among herbicides, several haloxyfop derivatives were found to demonstrate antimycobacterial activity via the inhibition of AccAD [45].

A malonyl-CoA:acyl carrier protein (ACP) transacylase (FabD) transfers the malonate group from malonyl-CoA to ACP (Figure 1). It is known that FabD is the target for the antibacterial action of aporphine alkaloids [46].

The malonyl-ACP, produced by FabD, is used by several β-ketoacyl-ACP synthases (KASs) of FASII: KAS I (FabB), KAS II (FabF), and KAS III (FabH). FabH initiates the cycle of elongation by condensing malonyl-ACP and acyl-CoA (Figure 1). The origin of the latter strongly depends on the bacteria. The huge variety in the fatty acid profile produced by different bacteria is defined by the substrate specificity of FabH. FabH of Escherichia coli is most specific for acetyl-CoA and propionyl-CoA and incapable of using branched-chain substrates and longer straight-chain acyl-CoA [47,48]. The specificity of Streptococcus pneumoniae FabH is significantly higher towards short (C₂–C₄) straight-chain than for branched-chain acyl-CoAs [6]. Rather than synthesizing unsaturated fatty acids (UFA) to fluidify the membranes (as Gram-negative bacteria and streptococci do), a number of Gram-positive bacteria (particularly Bacillus subtilis and Staphylococcus aureus) synthesize branched-chain fatty acids (BCFA) [2]. B. subtilis FabH displays higher efficiency with straight-chain and branched-chain acyl-CoA composed of C₂–C₄ compared to acetyl-CoA [48]. The substrate binding pocket of S. aureus FabH is substantially larger than that of E. coli FabH, and the activity of S. aureus FabH to elongate different acyl-CoAs decreases in the following order: isobutyryl → hexanoyl → butyryl → isovaleryl → acetyl-CoA [49]. The FabH homolog of M. tuberculosis, mFabH, to synthesize long-chain fatty acids (LCFA) prefers long-chain acyl-CoA substrates composed of C₂₀–C₄₀ rather than acetyl-CoA, short-chain, or branched-chain primers, due to the long internal acyl-binding channel [7,50]. Many FabH inhibitors have been discovered [51], and the dramatic variance in their activity against FabH from various stains clearly indicates the structural differences in the protein active sites. Thiolactomycin is known to inhibit BCFA and straight-chain fatty acid biosynthesis by targeting the FabH of Streptomyces coelicolor and Streptomyces glaucescens [52,53]. E. coli and S. pneumoniae FabH are weakly inhibited by thiolactomycin, while the indole compound 3-[(1,2-Benzisoxazol-3-yl) methoxy]benzamide, vanillic acylhydrazone, nitroimidazole, pyrazoline, and piperidine
derivatives, furoxan/sulfonylhydrazone hybrids, and others [54–75]. *S. aureus* FabH is weakly suppressed by thiolactomycin and is efficiently inhibited by different 1,2-dithiole-3-ones, 1,3,5-exadiazin-2-ones, and amycinin [76–78]. Selected benzoyleminobenzoic acid derivatives are also potent against FabH of *Enterococcus faecalis* and *Streptococcus pyogenes*, demonstrate only moderate activity against *S. aureus* FabH, and are ineffective against *H. influenzae* FabH [79–81]. Alkylsulfonyl compounds and pyrole-2-carboxylic acid derivatives are specific inhibitors of mfFabH [82–84]. Analyzing Table 1, one can conclude that SB418011 has the lowest IC₅₀ against FabH among the presented chemicals. It should be also noted that it did not demonstrate inhibitory activity against human FAS at 200-times higher concentrations [6]. Despite promising differences in selectivity, the in vivo efficacy and toxicity of SB418011 should be evaluated in further experiments.

Two other elongating KASs, FabB and FabF, operating later in the cycle, use acyl-ACP as the substrate for subsequent condensations, instead of the acetyl-CoA used by FabH [85] (Figure 1). The structural similarities between the active sites of FabB, FabF, and FabH [86–89] presume the development of antimicrobials that are able to hit several KASs at the same time, preventing the de novo synthesis of the fatty acids required for bacterial growth and survival. Two fungal products, thiolactomycin and cerulenin, are non-selective inhibitors of KASs, blocking FabB, FabF, and FabH with varying degrees of success [8,9,90,91]. It is found that cerulenin is characterized by its preferential selectivity towards FabB and FabF and is a very poor inhibitor of FabH (Table 1) [6–8,76]. The alteration in the inhibitory activity of cerulenin against various KASs is suggested to be a result of differences in the catalytic triads of the β-ketoacyl-ACP synthases and the size of the acyl-chain binding pockets [90]. Natural bacterial diterpenoid products platensimycin and platencin also target the KASs of FASII [92]. Platensimycin preferentially inhibits the chain elongation enzyme FabF, whereas platencin inhibits both chain initiation and elongation-condensing KASs, FabH and FabF (Table 1) [10–12,93–96]. According to the IC₅₀ information presented in Table 1, platensimycin is the most potent inhibitor of FabF. Moreover, it has great potential to inhibit methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococci* [97]. The low mammalian cell toxicity and the lack of antifungal activity indicate that platensimycin acts selectively [12,98]. This makes it extremely promising to search for new platensimycin-based antimicrobials in order to improve its pure pharmacokinetic properties. Thus, several semisynthetic analogs of platensimycin with enhanced in vivo efficacy towards MRSA infection in a mouse peritonitis model and improved pharmacokinetic properties have been developed [99,100]. In silico docking studies clearly demonstrated that the specific structural motif presented in *fasamycin* is predicted to be one more naturally occurring pharmacophore for the specific inhibition of FabF [101]. A series of *N*-substituted benzoazolinones were also shown to be active towards elongating KASs, FabB and FabF, by docking into the thiolactomycin binding site [102].

An NADPH-dependent β-ketoacyl-ACP reductase (FabG) produces the reduction of β-ketoacyl-ACP to β-hydroxyacyl-ACP. β-hydroxyacyl-ACP dehydrases (FabZ or FabA) and dehydrates β-hydroxyacyl-ACP to yield trans-2-enoyl-ACP. Another NADPH-dependent reductase of the FASII, trans-2-enoyl-ACP reductase (FabI), reduces trans-2-enoyl-ACP to form acyl-ACP, which can reenter the elongation cycle as a substrate for elongating KASs, FabB or FabF, or can be used for lipid production via phospholipid acyltransferases (PilsB/PilsC or PilsX/PilsY/PilsC system) [36] (Figure 1).

A broad range of plant polyphenols, including epigallocatechin gallate, gallatechin gallate, epicatechin gallate, catechin gallate, luteolin-7-O-glucoside, luteolin, quercetin, fisetin, morin, and myricetin, are potent inhibitors of all three crucial FASII enzymes, FabG, FabZ, and FabI (Table 1) [13,14,16]. Hexachlorophene and its anthelmintic bis-(2-hydroxyphenyl)methane/sulfide analogs are believed to exhibit antimalarial activity by inhibiting *Plasmodium falciparum* FabG [103]. Trans-cinnamic acid derivatives and macrolactins showed the inhibition of *E. coli* and *S. aureus* FabG (Table 1) [17,104].
Ethyl-6-bromo-2-((dimethylamino)methyl)-5-hydroxy-1-phenyl-1H-indole-3-carboxylate was demonstrated to be potent against Acinetobacter baumannii FabG [105]. A series of small-molecule Pseudomonas aeruginosa FabG inhibitors were identified [106]. According to Table 1, various catechin gallates demonstrate the most impressive activity (expressed as the lowest levels of IC$_{50}$) against three key FASII enzymes at once, compared to other inhibitors presented. The triple inhibiting action of these polyphenols, as well as their relatively low toxicity, has kept these naturally occurring compounds as the focus of researchers’ attention in terms of finding more safe antibiotics. For example, a safe intake level of green tea polyphenol epigallocatechin gallate, derived from toxicological and human safety data, is about 300 mg per day for adults [107]. We find another approach that involves combining natural polyphenols with other antibiotics that have alternative mechanisms of action on pathogen metabolism to be the most relevant [108–110].

FabZ is a crucial enzyme to elongate both saturated fatty acids (SFA) and UFA, and this is why it is an attractive target for the discovery of new antibacterials. The inhibitors of P. falciparum FabZ, NAS-21 (4,4,4-trifluoro-1-(4-nitrophenyl)-butane-1,3-dione), NAS-91 (4-chloro-2-[5-(chloroquinolin-8-5 yl)oxy]phenol), and their variants, were identified [111]. NAS-21 and NAS-91 analogs also demonstrated activity against mycobacterial FabZ (Table 1) [18]. The natural anthraquinone emodin (3-methyl-1,6,8-trihydroxyanthraquinone) and several synthetic inhibitors of Helicobacter pylori FabZ, based on two promising chemical scaffolds, namely (3,5-dibromo-2,4-dihydroxy-benzylidene)-hydrazide and 2-chloro-5-[3(2-methoxy-ethyl)-4-oxo-2-phenylimino-thiazolidin-5-ylidinemethyl]-furan-2-yl-benzoic acid, were discovered (Table 1) [20,112]. Two novel inhibitors of Francisella tularensis and Yersinia pestis FabZ, mangostin and stictic acid, have been found (Table 1) [19]. Moreover, 1,4-naphthoquinone and juglone (5-hydroxy-1,4-naphthoquinone) is a dual inhibitor of FabZ and FabD that might be potent against M. catarrhalis and H. pylori (Table 1) [21,22]. The therapeutic application of juglone is limited by its possible toxicity [113], but this pharmacophore might be used to find more safe and potent inhibitors of FabZ and FabD.

In Gram-negative bacteria, two enzymes, FabA and FabB, catalyze the production of UFA [114]. FabA has a dual function as a β-hydroxyacyl-ACP dehydratase, catalyzing the dehydration of β-hydroxyacyl-ACP to the trans-2-enoyl-ACP (as FabZ does), and as a trans-2-cis-3-decenoyl-ACP isomerase, producing the transformation of the trans-2-decenoyl-ACP to a cis-3-decenoyl-ACP (Figure 1). The size of tunnel in the active site of FabA, which perfectly fits trans-2-decenoyl-ACP, determines the specificity of the isomerization reaction at the 10-carbon stage of the unbranched substrate [115]. FabB specifically elongates the cis-UFA produced by FabA. FabA and FabB rival trans-2-decenoyl-ACP, and this balance determines the UFA/SFA ratio and the fluidity of the bacterial membrane [116]. S. pneumoniae and Streptococcus mutans have only one β-hydroxyacyl-ACP dehydrase (FabZ), while another enzyme, FabM, catalyzes the reaction of double-bond isomerization from trans-C$_2$=C$_3$ to cis-C$_2$=C$_3$ [117,118]. In E. faecalis, FabN performs the role of FabA, and FabF elongates the cis-UFA produced by FabN [119]. FabQ from Aerococcus viridans can act as a monofunctional dehydrase like FabZ or as a bifunctional dehydratase/isomerase like FabA (Figure 1) [120]. To produce UFA, some bacteria, particularly Bacillus subtilis and Pseudomonas aeruginosa, contain fatty acid desaturases, introducing a double bond into saturated acyl chains attached to phospholipids or acyl-CoA [114,121]. S. aureus does not contain FabA (or its analogs) or any desaturases, but can utilize the exogeneous UFA via acyl-ACP synthetase [36].

Furthermore, 3-decenoyl-N-acetyl cysteamine is a substrate-mimicking inhibitor of FabA, which covalently bonds to the active site of the enzyme [115]. N42FTA (3-(pyridin-2-yl oxy)aniline and N-(4-chlorobenzyl)-3-(2-furyl)-1H-1,2,4-triazol-5-amine) may be a promising scaffold to design more potent inhibitors of P. aeruginosa FabA [122,123].
Many compounds targeting FabI (including those undergoing clinical trials) are known: the front-line antituberculosis drug isoniazid, the common antiseptic triclosan (which demonstrates strong antimalarial activity via P. falciparum FabI inhibition), diazaborines, CG400462, CG400549, MUT056399, AFN-1252, AFN-1720, xanthorhizol, benzoxaboroles, and their derivatives (Table 1) [15,24,26–28,124–134]. FabI inhibition, in the cases of triclosan, CG400462, CG400549, MUT056399, AFN-1252, xanthorhizol, and benzoxaboroles, was validated by the isolation of resistant clones (Staphylococci and Chlamydia) containing mutations in the FabI gene [28,127,128,131,134,135]. A series of 2,9-disubstituted 1,2,3,4-tetrahydropyridro [3,4-b]indoles, 1,4-disubstituted imidazoles, 1-benzyl-1H-benzoimidazoles, and 4-pyridone and imidazole coumarin derivatives, as well as N-carboxy pyrrolidine analogs inhibiting S. aureus and/or E. coli FabI, were also reported [136–140]. Some natural macrocyclic compounds (complestatin, neuroprotectin, and chloropeptin), methyl-branched fatty acids (14-methyl-9(Z)-pentadecenoic and 15-methyl-9(Z)-hexadecenoic acids), meleagrin, phellinstatin, chelcomoracin, and moracin C demonstrated a promising ability to target S. aureus FabI (Table 1) [23,25,29,30]. In addition to the isoniazid, the trans-2-enoyl-ACP reductase from M. tuberculosis (InhA), which is involved in the biosynthesis of long-chain fatty acids (LCFA) (mycolic acids), was shown to be a target for several triclosan and benzodiaxborine derivatives; more specific inhibitors of InhA were also developed [141–150]. According to Table 1, triclosan- and complestatin-related compounds are characterized by similar low IC₅₀ values against S. aureus FabI. Taking into account that triclosan's application is limited by the possibility of bacterial resistance development via different mechanisms, including mutations in the genes of FabI or multidrug efflux pump [151–154] and significant cytotoxic effects [155], the search for natural macrocyclic compounds that are able to inhibit FabI seems to be a more promising approach to identify novel antibiotics.

It should be noted that four enoyl-ACP reductase isozymes have been reported in bacteria (Figure 1). S. pneumoniae, E. faecalis, and Clostridia have FabK instead of FabI, and FabV was discovered in Vibrio cholerae. Moreover, some pathogens have more than one enoyl-ACP reductase; for example, FabI and FabK in pseudomonads and enterococci, FabI and FabL in B. subtilis, and FabI, FabK, and FabV in P. aeruginosa [156–159]. Triclosan, inhibiting FabI, is a poor inhibitor of FabI and has no activity against FabK and FabV [157]. FabMG, isolated from the soil metagenome, was predicted to be a novel triclosan-resistant enoyl-ACP reductase, revealing that the main mechanism to develop triclosan resistance is a mutation of FabI [160]. Indole naphthyridinones and aquastatin A are inhibitors of both FabI and FabK [31,161], while AG205, atromentin, and leucemone are thought to be specific to FabK (Table 1) [32,162]. Carfilzomib showed high binding affinity with Klebsiella pneumoniae FabI and FabV [163]. Despite the fact that atromentin has a low IC₅₀ against S. pneumoniae FabK (Table 1), compounds that are able to inhibit various enoyl-ACP reductase isozymes (in particular, complestatin, aquastatin A, and carfilzomib) are more preferable for the development of broad-spectrum antibacterials.

The length of the hydrocarbon chains of the membrane lipids of bacteria is determined by competition between elongating KASs (FabF/FabB) and acyltransferases for acyl-ACP produced by trans-2-enoyl-ACP reductases (FabI/FabK/FabL/FabV). The upper limit is defined by the substrate specificity of the elongating KASs, while the lower limit is a result of the specificity of acyltransferases [164–166]. Glycerol-3-phosphate acyltransferases transfer two acyl chains from two acyl-ACPs to the 1 and 2 positions of glycerol-3-phosphate to produce phosphatidic acid (PA), a universal precursor of bacterial phospholipids (Figure 1). Two various acyltransferase systems, PlsX/PlsY/PlsC and PlsB/PlsC, have been discovered [167,168].

The more widespread bacterial pathway for the formation of PA involves the sequential transfer of acyl from acyl-ACP to acyl-phosphate (acyl-PO₄) via phosphate acyltransferase (PlsX) and then to the 1 position of glycerol-3-phosphate to produce lysophosphatidic acid (LPA) through acyl-phosphate:glycerol-3-phosphate
acyltransferase (PlsY) [164]. Acyl-PO₄ is a single substrate for PlsY; it cannot utilize acyl-ACP or acyl-CoA [168]. A series of stabilized acyl-phosphate mimetics, including acyl-phosphonates, reverse amide-phosphonates, and acyl-sulfamates, demonstrated promising activity against S. pneumoniae, Bacillus anthracis, and S. aureus through the inhibition of PlsY (Table 1) [33,34]. The lead compound, having a low IC₅₀ against S. pneumoniae PlsY, (Z)-1-oxooctadec-11-enylphosphoramidic acid, demonstrated potential toxicity [33]. These data necessitate a further search for ways to expand the therapeutic window of acyl-phosphate mimetics.

A glycerol-3-phosphate acyltransferase (PlsB) catalyzes the ligation of the acyl chain to the 1 position of glycerol-3-phosphate to produce LPA. A 1-acyl-sn-glycerol-3-phosphate acyltransferase (PlsC) ligates the second acyl chain (in the 2 position) to the LPA produced by PlsX/PlsY or PlsB to form PA. The advantage of the existence in bacteria of two distinct pathways, the PlsB/PlsC and PlsX/PlsY/PlsC acyltransferase systems, is the possibility of using exogenous fatty acids, because PlsB/PlsC might utilize not only the acyl-ACP produced by trans-2-enoyl-ACP reductases (FabI/FabK/FabL/FabV) but also the acyl-CoA thioesters derived from exogenous fatty acid metabolism—for example, those produced by FabD [168,169]. A fatty acid-rich environment in the host might facilitate the pathogen strain’s resistance to FASII inhibitors by enhancing the assimilation of exogenous fatty acids [170,171]. S. aureus can become insensitive to the FabI inhibitor triclosan via mutations in FabD, lowering FabD activity and inducing the integration of exogenous fatty acids [171,172]. The UFA-to-SFA ratio, membrane fluidity, and cell growth of Rhodobacter sphaeroides were reinstated upon both the inhibition of FabI with diazaborine and the introduction of exogenous UFA [173].

The cyclopropanation of fatty acids is intended to rigidify the bacterial membranes under stress conditions [174–178]. Cyclopropane fatty acids (CFA) are synthesized by cyclopropane fatty acid acyl-phospholipid synthase (CfaS) via the addition of a methylene group to the cis double bonds of the UFA chains of membrane phospholipids (Figure 1) [179]. Dioctylamine inhibits the CfaS of H. pylori (Table 1), preventing bacterial insensitivity to acid stress, antibiotics, and macrophage killing [35]. M. tuberculosis produces a number of cyclopropanated lipids, including mycolic acids, which are essential components to maintain cell wall integrity (Figure 1). This may indicate that agents capable of inhibiting the lipid cyclopropanation enzymes may be an approach to combatting tuberculosis pathogens [180,181].

2.1.2. Biosynthesis of Head Groups of Bacterial Lipids

In addition to the enzymes participating in the synthesis of the fatty acids of bacterial membrane lipids, there are unique enzymes that are involved in the synthesis of the heads of lipid molecules. Figure 2 summarizes the information about the synthesis of the lipid heads.
Figure 2. Schematic representation of the synthesis of “heads” of bacterial membrane lipids. The red, blue, and purple ellipses indicate that the enzyme is produced by Gram-positive, Gram-negative, or both Gram-positive and Gram-negative bacteria, respectively. Some examples of enzyme inhibitors are shown in a black box. Abbreviations: CdsA — cytidine diphosphate-diacylglycerol synthase; PgsA — phosphatidylglycerolphosphate synthase; PgpA, PgpB, and PgpC — phosphatidylglycerolphosphate phosphatases;ClsA, ClsB, ClsC, Cls1, and Cls2 — cardiolipin synthases; PssA — phosphatidyserine synthase; Psd — phosphatidyserine decarboxylase; Pcs — phosphatidylcholine synthase; PIS — phosphatidylinositol synthase; MprF — lysyl phosphatidylglycerol synthase and flippase (multiple peptide resistance factor); MdoB — phosphoglycerol transferase; DgkB and DgkA — diacylglycerol kinases; YfpP — diacylglycerol β-glucosyltransferase; PA — phosphatidic acid; CDP-DG — CDP-diacylglycerol; PS — phosphatidylserine; PE — phosphatidylethanolamine; PC — phosphatidylcholine; PI — phosphatidylinositol; PGP — phosphatidylglycerol phosphate; PG — phosphatidylglycerol; CL — cardiolipin; LPG — lysyl phosphatidylglycerol; DG — diacylglycerol; MGDG — monoglycosyl-DG; DGDG — diglycosyl-DG; R1 and R2 — fatty acid hydrocarbon radicals.

Cytidine disphosphate-diacylglycerol synthase (CdsA) is the critical enzyme catalyzing the production of the key intermediate in phospholipid diversity, CDP-diacylglycerol (CDP-DG), from cytidine triphosphate (CTP) and PA (Figure 2). CDP-DG is a precursor of the major phospholipids, including phosphatidylglycerol (PG), cardiolipin (CL), phosphatidylethanolamine (PE) (produced through the decarboxylation of phosphatidyserine (PS)), and even phosphatidylinositol (PI) and phosphatidylcholine (PC), which is absent in most prokaryotic cells. The fundamental nature of the CDP-DG-dependent pathway, characteristic of both prokaryotic and eukaryotic phospholipid biosynthesis [182,183], makes the majority of CDP-DG-converting enzymes poor targets for antibiotic therapy.

Phosphatidylglycerol phosphate (PGP) is synthesized by phosphatidylglycerolphosphate synthase (PgsA) from CDP-DG via the displacement of cytidine monophosphate (CMP) by glycerol-3-phosphate (glycerol-3-P) (Figure 2). Further, phosphatidylglycerolphosphate phosphatase (PgpA) dephosphorylates PGP to yield PG (Figure 2). Two additional genes of phosphatidylglycerolphosphate...
phosphatases, \textbf{PgpB} and \textbf{PgpC}, were discovered in \textit{E. coli} [184]. Subsequently, a cardiolipin synthase (\textbf{ClsA}) utilizes two PG molecules to produce \textit{CL} and \textit{glycerol} (Figure 2). Two extra \textit{CL} synthases have been discovered in \textit{E. coli}, \textbf{ClsB} and \textbf{ClsC} [185]. The first one can use one molecule of PG and the molecule of another phospholipid as the second substrate [186]. Moreover, \textbf{ClsB} of \textit{E. coli} can convert PE and \textit{glycerol} into PG in a \textbf{PgsA}-independent manner [187]. To form CL, \textbf{ClsC} uses PG and PE instead of the two PG molecules [185]. The products of the \textbf{Cls1} and \textbf{Cls2} genes of \textit{S. aureus} are \textit{CL} synthases with various types of stress-activated production [188]. Three genes of \textit{CL} synthases have been identified in \textit{B. subtilis} [189].

The phosphatidylserine synthase (\textbf{PssA}) synthesizes PS from CDP-DG via the displacement of CMP by serine (Figure 2). PS is only a minor biosynthetic intermediate in most bacteria and is decarboxylated by phosphatidylserine decarboxylase (\textbf{Psd}) to produce PE.

PC is also absent in most prokaryotic cells, although some Gram-negative bacteria contain phosphatidylcholine synthases (\textbf{Pcs}) to condense \textit{choline} into the phosphatidyl moiety of CDP-DG, similar to \textbf{PssA} with serine (Figure 2) [190,191].

One more component that is rarely present in bacterial membranes is PI. For example, \textit{Mycobacteria} are able to form PI using phosphatidylinositol synthase (\textbf{PIS}) via the exchange of the CMP moiety of CDP-PG for inositol (Figure 2) [192]. Due to the lack of sequence homology between bacterial and mammalian \textbf{PISs}, their different kinetic characteristics, and the essential role of PI in mycobacteria, the \textbf{PIS} of mycobacteria seems to be a good potential drug target for antimycobacterial therapy. Structural analogs of inositol were shown to be more potent inhibitors of mycobacterial \textbf{PIS} compared to mammalian \textbf{PIS} [193]. Alternatively, there is a difference in the bacterial and mammalian biosynthetic pathways used to form PI: in \textit{Mycobacteria}, PI is produced from CDP-DG and inositol 1-phosphate through an intermediate, phosphatidylinositol phosphate (PIP), which is dephosphorylated subsequently to PI, and inositol 1-phosphate analogs serving as inhibitors of \textbf{PIS} synthase can be used as antimycobacterials [194].

In some Gram-positive bacteria, the anionic glycerophospholipids, particularly PG and \textit{CL}, can be decorated with aminoacyl residues, most often with lysil, to form cationic PG and \textit{CL} derivatives by lysil phosphatidyglycerol (LPG) synthase and flippase, multiple peptide resistance factors (\textbf{MprF}) (Figure 2). This pathway is crucial for the adaptation of bacteria to cationic antimicrobial peptides [195–197]; for this reason, \textbf{MprF}-targeting antibodies or inhibitors of the factors involved in \textbf{MprF} regulation might sensitize resistant strains to antimicrobial agents [198,199].

In Gram-negative bacteria, a phosphoglycerol transferase (\textbf{MdoB}) transfers sn-1-phosphoglycerol from PG to membrane-derived oligosaccharides (MDO) to obtain diacylglycerol (DG). Further, it is phosphorylated by DG kinases (\textbf{DgkA} and \textbf{DgkB} in Gram-negative and Gram-positive bacteria, respectively) to generate PA, which can be recycled in the phospholipid biosynthetic pathway (Figure 2). \textbf{DgkA} presents a large family of prokaryotic DG kinases that are unrelated to the eukaryotic DG kinases and \textbf{DgkB} [200]. Some products of the \textbf{dgkA} gene are undecaprenol kinases [201].

In Gram-positive bacteria, DG is used to form glycolipids. A diacylglycerol \textit{β}-glucosyltransferase (\textbf{YpfP}) uses uridine diphosphate-glucose (UDP-Glc) to attach one monosaccharide unit to DG to form monoglycosyl-DG (MGDG) and to add one more Glc residue to MGDG to yield diglycosyl-DG (DGDG) (Figure 2). Although \textbf{YpfP} is a viable target for the development of novel antibacterial drugs [202], there are no approved inhibitors for this enzyme. Anionic glycopolymers, called lipoteichoic acids, composed of 1,3-polyglycerol-phosphate attached to DGDG (anchoring lipoteichoic acids in the membrane), are exposed on the cell walls of Gram-positive bacteria.

2.1.3. Biosynthesis of Lipid A

The outer leaflets of the outer membranes of Gram-negative bacteria are formed by specific lipopolysaccharides (LPS). They consist of O-antigen and core sugar domains
and a lipid anchor, known as lipid A. This phosphorylated disaccharide lipid is highly conserved and absolutely required for bacterial growth and survival [203,204]. For this reason, many enzymes involved in lipid A biosynthesis have been identified as targets for antibiotic development [205] (Figure 3, Table 2).

Figure 3. Schematic representation of the biosynthesis of lipid A. The blue ellipses indicate that all enzymes are only produced by Gram-negative bacteria. Some examples of enzyme inhibitors are shown in a black box. Abbreviations: LpxA — UDP-N-acetylglucosamine acyltransferase; LpxC — UDP-3-O-(R-3-hydroxyacyl)-N-acetylglucosamine deacetylase; LpxD — UDP-3-O-(R-3-hydroxyacyl)glucosamine N-acyltransferase; LpxH, LpxL, and LpxP — UDP-diacylglucosamine pyrophosphohydrolases; LpxB — lipid-A-disaccharide synthase; LpxK — tetraacyldisaccharide-1-phosphate 4'-kinase; WaaA — 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo) transferase; LpxL, LpxM, and LpxP — lysophospholipid acyltransferases.

Table 2. Major inhibitors of lipid A biosynthetic pathway.

<table>
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<tr>
<th>Inhibitor</th>
<th>Structure</th>
<th>Enzyme</th>
<th>Origin</th>
<th>IC_{50}, μM</th>
<th>References</th>
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<td></td>
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<td>[208]</td>
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<td>[210]</td>
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</tbody>
</table>
A UDP-N-acetylglucosamine acyltransferase (LpxA) induces the first step of lipid A biosynthesis (Raetz pathway). It transfers a β-hydroxyacyl chain from β-hydroxyacyl-ACP generated by FabG to the 3 position of UDP-N-acetyl-glucosamine (UDP-GlcNAc) (Figure 3). It should be noted that LPS-producing enzymes are highly selective towards ACP thioesters; they cannot be substituted by normal fatty acids [220]. LpxA enzymes are highly specific regarding the acyl chain length. For example, *E. coli* LpxA transfers only β-hydroxymyristoyl chains [221]. Peptide (Peptide 920, RJPXD33) and small-molecule inhibitors (particularly (R)-(3-(2-chloro-6-methoxybenzyl)morpholino)(3-(4-methylpyridin-2-yl)-1H-pyrazol-3-yl)methanone and erythroskyrin) of LpxA were reported to compete with the substrate or interact with the complex product (Table 2) [206–208,222–227]. Analyzing Table 2, one can conclude that peptide 920 is of interest due to its relatively low IC₅₀ value, while RJPXD33 demonstrates dual targeting of LpxA and LpxD, offering the possibility to develop novel dual-binding antimicrobials. However, systematic studies of the safety of the peptide's...
administration must be performed before it can be determined how promising these methods are.

The acyl transfer reaction by \textbf{LpxA} is thermodynamically reversible and unfavorable, and the subsequent second reaction of the Raetz pathway, catalyzed by UDP-3-O-(R-3-hydroxyacyl)-N-acetylglucosamine deacetylase (\textbf{LpxC}), should occur (Figure 3). \textbf{LpxC} splits the acetyl radical from the UDP-3-(β-hydroxyacyl)-N-acetylglucosamine to produce UDP-3-(β-hydroxyacyl)-D-glucosamine (acyl-UDP-GlcN). Small-molecule inhibitors of the \textbf{LpxC} have been discovered, including hydroxamate-based compounds, exemplified by TU-514, BB-78484, BB-78485, L-159,692, L-161,240, L-573,655, CHIR-090, LPC-009, LPC-011, and LpxC-4 (Table 2) [209–215,228–234]. Some of them are highly potent and have proven to be active against various multidrug-resistant Gram-negative bacteria. Analyzing Table 1, it can be assumed that the greatest interest regarding the design of new antibacterials targeting \textbf{LpxC} is in the further optimization of the most effective compounds, L-161,240, CHIR-090, and LpxC-4, in order to avoid emerging resistance [214,234–237].

A UDP-3-O-(R-3-hydroxyacetyl)glucosamine N-acyltransferase (\textbf{LpxD}) performs the third reaction of the lipid A biosynthetic pathway; it transfers a second acyl group from β-hydroxyacyl-ACP to acyl-UDP-GlcN to produce UDP-2,3-bis(β-hydroxyacyl)-D-glucosamine (Figure 3). Some \textbf{LpxA} inhibitors, particularly \textbf{RJPX33}, also bind to and inhibit \textbf{LpxD} [207,222,224]. It is also believed that \textbf{LpxD} is a drug target of natural compounds like curcumin, gallocatechin, isoorientin, neral, isovitexin, vitexin, allicin, ajone, and cinnamaldehyde [237]. Several synthetic compounds related to hydro-pyrazolo-quinolinones were identified as \textbf{LpxD} inhibitors (Table 2) [216].

A UDP-diacylglucosamine pyrophosphohydrolase (\textbf{LpxH}) hydrolyses UDP-2,3-bis(β-hydroxyacyl)-D-glucosamine to split UMP and to generate 2,3-diacylglucosamine-1-phosphate (lipid \textit{X}) (Figure 3). \textbf{LpxI} and \textbf{LpxG} are functional orthologs of \textbf{LpxH} in α-proteobacteria and in Chlamydiae, respectively [238–240]. \textbf{LpxH} is inhibited by sulfonyl piperazine antibiotics (such as AZ1, JH-LPH-28, JH-LPH-33) (Table 2) [217–219,241–243]. Bacterial efflux pump functioning was found to be a significant deterrent for \textbf{LpxH}-targeting antimicrobials, highlighting the significance of their combination with antibiotics, permeabilizing the outer membrane to fight multidrug-resistant Gram-negative pathogens [218].

A lipid-A-disaccharide synthase (\textbf{LpxB}) combines the substrate and the product of the \textbf{LpxH}-catalyzed reaction to form the lipid A disaccharide (Figure 3). Compounds that target \textbf{LpxB} have not been discovered to date; only antisense pPNA technology is used to block the \textbf{lpxB} gene [244].

A tetraacyldisaccharide-1-phosphate 4′-kinase (\textbf{LpxK}) translocates the gamma-phosphate of ATP to the 4′ position of the lipid A disaccharide to produce lipid \textit{IVA} (Figure 3). The 5-(4-carbamoylbenzenesulfonyl)-N-hydroxy-1H-imidazole-2-carboxamide analogs (STOCK6S-33288, 35740, 37164, 39892, and 43621) are believed to be a promising template to develop novel potent \textbf{LpxK} inhibitors [245].

A 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo) transferase (\textbf{WaaA/KdtA}) adds two Kdo residues to lipid \textit{IVA} to form Kdo₂-lipid \textit{IVA} (Figure 3). Lyosphospholipid acyltransferases, \textbf{LpxL} and \textbf{LpxM}, incorporate two additional acyl chains at positions 2′ and 3′ of Kdo₂-lipid \textit{IVA} to yield a hexa-acylated Kdo₂-lipid A (Figure 3). At lower temperatures, \textbf{LpxP} might partially perform the function of \textbf{LpxL}. The structure of the active sites of \textbf{LpxA} and \textbf{LpxD} permits the incorporation of myristoyl residues, while acyltransferases \textbf{LpxL}, \textbf{LpxP}, and \textbf{LpxM} transfer lauroyl, palmitoleoyl, and myristoyl chains, respectively [246,247]. \textbf{LpxN} is an ortholog of \textbf{LpxM} in \textit{V. cholerae} [248]. No information about the specific compounds inhibiting the enzymes in the last steps of LPS biosynthesis are available in the literature.
2.2. Agents with Direct Action on Bacterial Lipid Membranes

Figure 4 summarizes the major mechanisms of the direct action of antibacterial agents on target lipid membranes. The mechanisms include pore formation and a detergent-like manner of action [249]. In the first case, the bacterium dies due to a violation in the water–salt balance via the formation of unauthorized transport pathways for water, ions, and small organic molecules. In the second case, the cause of death is the destruction of the membrane after reaching a critical detergent concentration, and a dramatic enhancement in the membrane fluidity and micellization of membrane lipids.

Antimicrobial peptides are synthetized as components of the immune system in higher eukaryotes to defend them against a wide variety of invasive pathogens [250,251]. Antimicrobial lipopeptides are produced in bacteria or fungi as metabolites and/or to gain a competitive advantage over other species. A number of natural antimicrobial agents exert their defending activities primarily via pathogens’ membrane disruption due to pore formation or the disordering of membrane lipids, and they are characterized by a lower probability of inducing microbial resistance. Thus, owing to the high efficiency of these compounds, their broad-spectrum bactericidal effects, and the low rate of pathogens’ resistance to them, the use of antimicrobial peptides and lipopeptides in clinical practice, as well as in the search for new “natural” antibiotics, seems to be a productive anti-infective therapeutic strategy [252,253]. As a rule, antimicrobial peptides and lipopeptides share common structural features, such as molecular amphiphilicity and a net-positive electrical charge, which govern the binding and permeabilization of the negatively charged bacterial membranes through the mechanisms indicated above.

Table 3 presents examples of natural antimicrobial peptides and lipopeptides, their possible lipid targets, and the threshold concentrations needed to form pores and disintegrate lipid bilayers, mimicking the membranes of sensitive bacteria. Most of the antimicrobial peptides—gramicidin A from Bacillus brevis; alamethicin produced by the fungus Trichoderma viride; pardaxin isolated from secretions of the Red Sea Moses sole; melittin and mastoparan isolated from bee and wasp venom, respectively; protegrin-1 found in porcine leukocytes; magainin found in frog skin; ceratotoxins and cecropins
discovered in the accessory gland secretion fluid of the insect *Ceratitis capitata* and the hemolymph of *Hyalophora cecropia*, respectively; nisin from *Streptococcus lactis*; cinnamycin and its close analog duramycin from *Streptomyces* sp.; mammalian defensins; human cathelicidin LL-37 [254–274]; lipopeptides; colistins (polymyxins) from *Bacillus polymyxa*; daptomycin from *Streptomyces roseosporus*; and gausemycin from *Streptomyces* sp. [108,275–279]—manifest their action via the pore formation mechanism (Table 3). The pores formed by antimicrobial agents are characterized by their different architectures [280]. For example, alamethicin, pardaxin, and serotoxin A pores are believed to be “barrels” composed of peptide aggregates [271,272,281,282], while mellitin, magainin, and polymyxin B form (lipo)peptide–lipid toroidal pores [108,270,283–285] (Figure 4).

Some antimicrobial agents are not shown to form transmembrane pores; they act as detergents by forming a peptide “carpet” on the membrane surface (Figure 4). Such properties are exhibited by cecropin P1, lasioglossin III, and aurein 1.2 (Table 3) [264,286–288]. The peptide thanatin disrupts the bacterial outer membrane [289]. In the case of cecropins and protegrins, dual activity was found, including pore formation and detergent-like model action [259,264,270,290]. In any case, it is likely that when a critical antibiotic concentration is reached, regardless of whether the agent can form pores and in what way, an irreversible change in the rheological properties of the lipid bilayer occurs and it will be destroyed [291,292] (Figure 4).

**Table 3.** The effects of antibacterial agents on the model lipid membrane’s permeability.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Structure</th>
<th>$C_{\text{min}}$, $\mu$M</th>
<th>$C_{\text{tr}}$, $\mu$M</th>
<th>Lipid Composition</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pore formation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gramicidin A</td>
<td><img src="image" alt="Structure" /></td>
<td>0.001</td>
<td>*</td>
<td>DSPC</td>
<td>[254]</td>
</tr>
<tr>
<td>alamethicin</td>
<td><img src="image" alt="Structure" /></td>
<td>0.1</td>
<td>*</td>
<td>DOPS:DOPS 1:1 (m/m)</td>
<td>[293]</td>
</tr>
<tr>
<td>pardaxin</td>
<td><img src="image" alt="Structure" /></td>
<td>0.006</td>
<td>*</td>
<td>soybean lecithin</td>
<td>[258]</td>
</tr>
<tr>
<td>melittin</td>
<td><img src="image" alt="Structure" /></td>
<td>0.23</td>
<td>*</td>
<td>POPC:cholesterol 3:1 (m/m)</td>
<td>[294]</td>
</tr>
<tr>
<td>magainin I</td>
<td><img src="image" alt="Structure" /></td>
<td>10</td>
<td>*</td>
<td>DOPS:DOPS 3:1 (m/m)</td>
<td>[260]</td>
</tr>
<tr>
<td>magainin II</td>
<td><img src="image" alt="Structure" /></td>
<td>0.08</td>
<td>*</td>
<td>POPC:POPG 6:1 (m/m); DOPS:ergosterol 3:1 (m/m); POPC:ergosterol 3:1 (m/m)</td>
<td>[261]</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>Peptidomimetic Structure</td>
<td>Antimicrobial Activity</td>
<td>Membrane Model</td>
<td>References</td>
<td>Other References</td>
</tr>
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<td>--------------------------</td>
<td>------------------------</td>
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<td>-----------------</td>
</tr>
<tr>
<td>mastoparan</td>
<td><img src="mastoparan.png" alt="mastoparan" /></td>
<td>0.68 – * DPhPC</td>
<td></td>
<td>[262]</td>
<td></td>
</tr>
<tr>
<td>ceratotoxin A</td>
<td>![ceratotoxin A](ceratotoxin A.png)</td>
<td>0.02 – * POPC:DOPE 7:3 (w/w); POPC:DOPE:POPS 7:3:1 (w/w)</td>
<td></td>
<td>[271,272]</td>
<td></td>
</tr>
<tr>
<td>protegrin-1</td>
<td><img src="protegrin-1.png" alt="protegrin-1" /></td>
<td>0.25–10 – * DOPC:DOPE 1:1 (m/m)</td>
<td></td>
<td>[259]</td>
<td></td>
</tr>
<tr>
<td>nisin</td>
<td><img src="nisin.png" alt="nisin" /></td>
<td>~40 &gt;500 TOCL</td>
<td></td>
<td>[295]</td>
<td></td>
</tr>
<tr>
<td>cinnamycin</td>
<td><img src="cinnamycin.png" alt="cinnamycin" /></td>
<td>~1.5 &gt;10 DOPC; TOCL</td>
<td></td>
<td>[265]</td>
<td></td>
</tr>
<tr>
<td>duramycin</td>
<td><img src="duramycin.png" alt="duramycin" /></td>
<td>~2 &gt;12 DOPC; TOCL</td>
<td></td>
<td>[265]</td>
<td></td>
</tr>
<tr>
<td>rabbit α-defensins</td>
<td>![rabbit α-defensins](rabbit α-defensins.png)</td>
<td>~1 &gt;16 PE/PC/PS 2:2:1 (w/w); PE/CL</td>
<td></td>
<td>[296,297]</td>
<td></td>
</tr>
<tr>
<td>daptomycin</td>
<td><img src="daptomycin.png" alt="daptomycin" /></td>
<td>6.2 – * DPhPG</td>
<td></td>
<td>[298]</td>
<td></td>
</tr>
<tr>
<td>polymyxin B</td>
<td>![polymyxin B](polymyxin B.png)</td>
<td>2.5 &gt;100 DOPG</td>
<td></td>
<td>[108]</td>
<td>Kdo2-Lipid A</td>
</tr>
<tr>
<td>gayseqycin</td>
<td><img src="gayseqycin.png" alt="gayseqycin" /></td>
<td>~26 – * DOPG</td>
<td></td>
<td>[279]</td>
<td></td>
</tr>
</tbody>
</table>

**Pore formation and detergent action**

| Cecropin A          | ![cecropin A](cecropin A.png) | 1 >5 DOPS:DOPE 1:1 (m/m) | | [264] | |
| Cecropin B          | ![cecropin B](cecropin B.png) | 1 >5 DOPS:DOPE 1:1 (m/m) | | [264] | |
Despite the lower bacterial resistance to the naturally occurring antibiotics acting on the microbial membranes compared to classical antibiotics, including those inhibiting lipid biosynthesis, antimicrobial peptides and lipopeptides are not a panacea for the emergence of resistance in pathogenic bacteria. One of the evolutionary mechanisms by which to develop pathogenic resistance to cationic antibacterial agents is a reduction in the total negative charge of the cell surface of a microorganism to reduce the initial electrostatic binding. Thus, the resistance of *S. aureus* to defensins and protegrins is determined by the activity of MprF, an enzyme that modifies phosphatidylglycerol with L-lysine (1.1.2), which, in turn, leads to a decrease in the surface membrane charge and the repulsion of cationic peptides [299]. *Pseudomonas fluorescens* was proposed to diminish the net anionic charge of the cytoplasmic membrane by reducing the content of anionic phospholipids and increasing the concentration of positively charged ornithine–amide lipids that lead to the resistance to the cationic polymyxin B [300]. According to the literature data, a change in the structure of the LPS of Gram-negative bacteria *E. coli*, *Salmonella enterica*, *Salmonella typhimurium*, *K. pneumoniae*, and *P. aeruginosa* induced by the attachment of L-arabinose or phosphatidylethanolamine to the phosphate residues of lipid A leads to the emergence of resistance among these microorganisms to polymyxins due to changes in the membrane surface charge [301–305]. In turn, daptomycin is recommended for application as a therapy against β-lactam-resistant *Streptococcus mitis*. The target of daptomycin is thought to be phosphoglycerol [306]. However, *S. mitis* can rapidly develop resistance to daptomycin via loss-of-function mutations in the gene of CdsA, which catalyzes the formation of a common phospholipid precursor, CDP-DG (1.1.2); moreover, daptomycin-resistant strains exhibit the absence of anionic phospholipid membrane microdomains composed of CL and PG [307]. Daptomicin resistance in *E. faecalis* was found to be associated with changes in the genes of cardiolipin synthase, Cls, and cyclopropane fatty acid synthase, CfaS (1.1.2) [308]. The latter indicates that reducing the level of negatively charged lipids is not the only strategy for...
resistance development by changing the membrane properties; the fatty acid profile is also of fundamental importance. For example, the development of resistance of *S. aureus* to gausemycin A is accompanied by growth in the ratio between the levels of anteiso- and iso-BCFA [309]. The membrane fluidity is significantly enhanced when anteiso acyl chains replace iso acyl chains. In contrast, the resistance of *S. aureus* to daptomycin and *Listeria monocytogenes* to nisin develops with an increase in the percentage of SFA compared to BCFA, which should lead to a decrease in membrane fluidity [310,311]. Thus, alterations in the fatty acid profile and rheological properties of the membrane may be another important factor determining the sensitivity of pathogens to antibiotics. Moreover, whether the fluidity of the membrane should be increased or decreased depends on the architecture of the pores formed by a specific antimicrobial agent.

3. Antifungal Agents with Lipid-Related Mechanisms of Action

3.1. Inhibition of Biosynthesis of Fungal Cell Membrane Components

Fundamentally, fungal walls are all engineered in a similar way and contain the cell membrane and cell wall [312]. The absence of a cell wall in mammalian cells provides an opportunity for the development of antifungal agents that target the enzymes involved in the biosynthesis of cell wall components in fungi, chitin synthase (Chs) and β-1,3-glucan synthase (Fks) [313–315]. The resistance of *Aspergillus fumigatus* and *Candida glabrata* to semisynthetic echinocandin and caspofungin might arise from not only mutations in the Fks gene but also from alterations in the lipid microenvironment of the enzyme due to an increase in dihydrospinososine and phytosphingosine content [316,317]. Thus, it should be taken into account that although the cell wall is an essential structure, maintaining the integrity and viability of fungal cells, the fungal lipid membrane serves as both a second barrier and a platform for the functioning of the enzymes that are responsible for the cell wall's biosynthesis (Chs and Fks) [318,319]. The fungal cell membrane is composed of various glycerophospholipids, sphingolipids, and ergosterol. The latter component is interesting in terms of antifungal targeting, since mammalian cell membranes include another sterol, cholesterol.

3.1.1. Biosynthesis of Fatty Acids of Fungal Membrane Lipids

Recently, a discrepancy between the human and fungal FASII has been discovered [320]. The human FAS encoded by the FASN gene is a type Ib FAS. It consists of one polypeptide chain, including seven domains that assemble into homodimers [321]. Yeast FAS belongs to type Ia FAS and includes a heterododecameric complex composed of six subunits α and six subunits β, which are encoded by the genes Fas1 and Fas2 [322]. It was shown that the deletion of the FAS genes in *Cryptococcus neoformans* significantly reduced the growth and virulence of the fungi [323–325]. Thus, the differences in fungal and human FAS [320] can, in *Candida albicans*, potentially be used to target broad-spectrum antifungals towards the products of the Fas1 and Fas2 genes. However, the fungal mutants for the corresponding FAS genes could survive due to the utilization of exogenous fatty acids [326], which might significantly reduce the possibilities of anti-FAS therapy. There have been few fruitful efforts to repurpose antibacterial FAS inhibitors. FAS inhibition in *C. neoformans* with the FASII inhibitor cerulenin (1.1) drastically reduced the inhibitory concentration of the inhibitor of ergosterol synthesis, fluconazole (2.4) [323]. *Cerulenin* (but not platensimycin and thiolactomycin) was shown to inhibit Saccharomyces cerevisiae FAS [327]. The attempts to inhibit a product of the *OLE1* gene, fatty acid Δ9 desaturase, were more successful in terms of targeting fatty acid biosynthesis in *C. albicans* [328,329].

3.1.2. Biosynthesis of Phospholipid Head Groups

Similar to bacteria (Figure 2), the biosynthesis of the fungal phospholipids begins with the common precursor CDP-DG, which is produced from FA by CdsA [330,331].
Further, PI, PGP, and PS are generated from CDP-DG by **PIS**, **PgsA**, and **PssA**, respectively [330,332]. PGP is dephosphorylated by **PgpA** to form PG, and PG is condensed to CL by **ClsA** [330,332]. It is important that the major phospholipid present in most eukaryotic membranes is PC, and PS is a key substrate for PC synthesis in yeast and fungi [333]. The **Psd** enzyme converts PS to PE. The main pathway for PC synthesis in yeast involves the three-step methylation of PE (Figure 5). The first stage includes the methylation of PE by phosphatidylethanolamine N-methyltransferase (**Pems**) to form the phosphatidyl-N-monomethylethanolamine (PMME) and the methylation of PMME to form phosphatidyl-N,N-dimethylethanolamine (PDME) [330]. PDME is converted to PC by **PgpA**. It was shown that the disruption of PS and PE biosynthesis within the CDP-DG pathway causes the avirulence of *C. albicans* [334]. Moreover, the action of some fungicides is associated with **Pems** inhibition [335]. As, in the cells of higher eukaryotes, PC is mainly synthesized from exogenous ethanolamine and choline via the Kennedy pathway [336,337] (Figure 5), one might suggest that the enzymes that perform the methylation reactions in PC biosynthesis by the CDP-DG pathway can be potential targets for antifungals. However, the alternative Kennedy pathway can be used by lower eukaryotes to produce PC and determines the possibility of developing resistance to the action of such antibiotics.

**Figure 5.** The PC biosynthesis in *S. cerevisiae*. The de novo and Kennedy pathways are represented by the grey and violet lines, respectively. The enzymes of the indicated pathways are highlighted with green and light-green ellipses, respectively. Abbreviations: **Pems**—phosphatidylethanolamine N-methyltransferase; **EK**—ethanolamine kinase; **CK**—choline kinase; **ECT**—phosphoethanolamine cytidylyltransferase; **CCT**—phosphocholine cytidylyltransferase; **EPT**—ethanolaminephosphotransferase; **CPT**—cholinephosphotransferase; **PMME**—phosphatidyl-N-monomethylethanolamine; **PDME**—phosphatidyl-N,N-dimethylethanolamine.
3.1.3. Biosynthesis of Sphingolipids

Figure 6 demonstrates the pathway for the synthesis of sphingolipids in *S. cerevisiae* [338–340]. Serine palmitoyltransferase (SPT) performs the condensation of L-serine and palmitoyl-CoA to lead to 3-ketodihydrosphingosine [341]. Meanwhile, 3-ketodihydrosphingosine reductase (KDSR) converts 3-ketodihydrosphingosine to dihydrosphingosine. Phytoceramide can be synthesized from dihydrosphingosine by ceramide synthase (CerS) and sphingosine C1-hydroxylase (SCH) through two alternative intermediates, ceramide and phytosphingosine (Figure 6). The next reaction involves inositol-phosphoceramide synthase (IPCS), which converts phytoceramide to inositolphosphatidyl-ceramide (IPC). IPC can be further mannosylated by mannosylinositol phosphorylceramide synthase (MIPCS) and condensed with additional inositolphosphate using inositolphosphotransferase (IPS) to yield the more complex sphingolipids MIPC and M(IP)_2C, respectively [341–343].

Comparing the enzymes in the fungal and mammalian cells required for sphingolipid biosynthesis, it can be concluded that they are homologous and, consequently, are not suitable as targets for the development of low-toxicity antifungal drugs. Nevertheless, potent inhibitors of fungal sphingolipid biosynthesis are described in the literature, although a proper assessment of their possible toxicity has not been completed yet. Mainly, the compounds target SPT and IPCS. Table 4 summarizes the available information about the agents targeting the synthesis of sphingolipids in different fungi, their chemical structures, and the IC₅₀ values against appropriate fungal enzymes. In particular, a variety of different SPT inhibitors have been isolated, including lipoxamycin [344,345], myriocin [346], sphingofungins [347–349], and viridiofungins [350]. It is interesting that sphingofungins are characterized by similar activity against *C. albicans* and *S. cerevisiae* SPT, while viridiofungins show 70-200-fold higher selectivity towards *C. albicans* SPT (Table 4). Unfortunately, SPT inhibitors demonstrated high toxicity towards mammalian cells due to the inhibition of human SPT1 [351]. Interestingly, the *S. cerevisiae* SPT is composed of three different subunits, known as Lcb1, Lcb2, and Tsc3, and a homologue of Tsc3 has not been found in mammals [352]. Thus, the question of whether Tsc3 inhibition would be sufficient to effectively suppress the fungal SPT activity in pathogenic fungi awaits elucidation. Fumonisins are effective inhibitors of CerS, but also demonstrate toxicity to mammalian cells [353]. *Australifungin* is a very potent inhibitor of fungal CerS from several species; however, the α-diketone and β-ketoaldehyde functional groups present in this compound have high chemical reactivity, which seriously limits *australifungin*'s use [348]. The inositol-phosphoceramide synthase (IPCS) identified in *S. cerevisiae* and other different fungi [354] is a potential target for antifungals. IPCS inhibitors include *aureobasidin A* [355], *khafrefungin* [356], *haplofungins* [357], and *pleofungin* [358]. Galbolodine A and B inhibit the IPCS of *B. cinerea* and *C. neoformans* [359–361]. An analysis of Table 4 shows that *khafrefungin*, *pleofungin A*, and *galbonolide A*, also known as *rustmicin*, are the most potent inhibitors of *C. albicans*, *A. fumigatus*, and *C. neoformans* IPCS, respectively. *Khafrefungin* seems to be a more promising candidate due to its relevant selectivity between fungal and mammalian IPCS [356,362], while *rustmicin*'s application is limited by its low metabolic stability and drug efflux in fungi [359]. Thus, a further search may accelerate the discovery of selective low-toxicity natural inhibitors for fungi.
Figure 6. Schematic representation of the sphingolipid biosynthetic pathways in *S. cerevisiae*. The enzymes are highlighted with orange ellipses. Some examples of enzyme inhibitors are shown in black boxes. Abbreviations: SPT—serine palmitoyltransferase; KDSR—3-ketodihydrophosphogingosine reductase; CerS—ceramide synthase; SCH—sphingosine C4-hydroxylase; IPCS—inositol-phosphoceramide synthase; MIPCS—mannosylinositol phosphorylceramide synthase; IPS—inositolphosphotransferase; IPC—inositolphosphoceramide; MIPC—mannose-inositol-phosphoceramide; M(IP)2C—mannose-(inositol-P)2-ceramide.

Table 4. Major inhibitors of fungal sphingolipid biosynthesis.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Structure</th>
<th>Enzyme</th>
<th>IC50, μM</th>
<th>References</th>
</tr>
</thead>
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<td>sphingofungin B</td>
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<td>SPT</td>
<td>C. albicans 0.049</td>
<td>[350]</td>
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<td></td>
<td></td>
<td></td>
<td>S. cerevisiae 0.051</td>
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<tr>
<td>viridiofungin A</td>
<td><img src="image2" alt="Structure" /></td>
<td>SPT</td>
<td>C. albicans 0.022</td>
<td>[350]</td>
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<td></td>
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<td>S. cerevisiae 4.7</td>
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<tr>
<td>viridiofungin B</td>
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<td>SPT</td>
<td>C. albicans 0.017</td>
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<td>S. cerevisiae 1.68</td>
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</table>
### 3.1.4. Ergosterol Synthesis

Contrary to the cholesterol-containing membranes of mammalian cells, fungal cell membranes are enriched with ergosterol [364]. Figure 7 demonstrates the ergosterol biosynthesis pathway in *S. cerevisiae* [365].

The ergosterol biosynthesis in *S. cerevisiae* includes three different modules, mevalonate, farnesyl-PP, and ergosterol biosynthesis [366]. Table 5 provides the available information about the potential inhibitors of enzymes participating in the ergosterol biosynthetic pathway.

Acetyl-CoA C-acetyltransferase (ERG10) catalyzes the additional acetylation of acetyl-CoA molecules to produce acetoacetyl-CoA, which is further transformed by hydroxymethylglutaryl-CoA synthase (ERG13) to 3-hydroxy-3-methylglutaryl-CoA. Mevalonate is synthesized by NADPH-dependent hydroxymethylglutaryl-CoA reductase (HMG1/2) [366,367]. As the synthesis of mevalonate is the critical step in the ergosterol biosynthetic pathway, it is believed that HMG1/2 might be a good target for antifungals. It is well known that statins competitively bind to human 3-hydroxy-3-methylglutaryl coenzyme-A reductase, preventing the conversion of 3-hydroxy-3-methylglutaryl-CoA into mevalonate [368,369], and the prospects for the repurposing of statins to treat fungal infections should be estimated. Supporting this theory, simvastatin, lovastatin, atorvastatin, pravastatin, fluvastatin, and related compounds were reported to decrease the intracellular ergosterol level via the inhibition of HMG1/2 in *C. glabrata*, *C. albicans*, *Ustilago maydis*, *Trichothecium roseum*, *S. cerevisiae*, *C. neoformans*,

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC (μg/mL)</th>
<th>Source</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>aureobasidin A</strong></td>
<td></td>
<td>IPCS</td>
<td><em>C. albicans</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>C. glabrata</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Candida tropicalis</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Candida parapsilosis</em></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Candida krusei</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>A. fumigatus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Aspergillus flavus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Aspergillus terreus</em></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.004</td>
</tr>
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<td></td>
<td></td>
<td><em>Aspergillus niger</em></td>
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<td>0.004</td>
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<tr>
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<td></td>
<td></td>
<td><em>S. cerevisiae</em></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.0009</td>
</tr>
<tr>
<td><strong>khafrefungin</strong></td>
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<td>IPCS</td>
<td><em>C. albicans</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0006</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>C. neoformans</em></td>
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<tr>
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<td></td>
<td></td>
<td>0.031</td>
</tr>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.007</td>
</tr>
<tr>
<td><strong>haplofungin A</strong></td>
<td></td>
<td>IPCS</td>
<td><em>S. cerevisiae</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0025</td>
</tr>
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<td><em>A. fumigatus</em></td>
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<td><strong>haplofungin B</strong></td>
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<td>IPCS</td>
<td><em>S. cerevisiae</em></td>
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<td>0.042</td>
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<td><em>A. fumigatus</em></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1.33</td>
</tr>
<tr>
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<td></td>
<td><em>S. cerevisiae</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.007</td>
</tr>
<tr>
<td><strong>pleofungin A</strong></td>
<td></td>
<td>IPCS</td>
<td><em>A. fumigatus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0009</td>
</tr>
<tr>
<td><strong>galbonolide A</strong></td>
<td></td>
<td>IPCS</td>
<td><em>C. albicans</em></td>
</tr>
<tr>
<td>(rustmicin)</td>
<td></td>
<td></td>
<td>0.0038</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>C. neoformans</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.00007</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>S. cerevisiae</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0198</td>
</tr>
</tbody>
</table>
Zygomycetes, and Aspergillus spp. [370–381]. Moreover, statin therapy is associated with a reduction in oral Candida carriage in hyperlipidemic patients [382]. Statins also reduced mortality due to the diminishing risk of fungal-related complications in patients with diabetes, hematologic malignancies, and COVID-19 [368,383–385]. Further, mevalonate is successively phosphorylated to mevalonate-PP by two different kinases, mevalonate kinase (ERG12) and phosphomevalonate kinase (ERG8). Diphosphomevalonate decarboxylase (ERG19) performs the transformation of mevalonate-PP to isopenenyl-PP [386]. Farnesyl-PP is a product of two successive reactions catalyzed by farnesyl diphosphate synthase (ERG20) [387].

Using NADPH, squalene synthase (ERG9) produces squalene from farnesyl-PP. Natural fungal metabolites, such as zaragozic acids, are potent inhibitors of ERG9 [388]; however, due to their high toxicity, the compounds failed to reach the clinical trial phase [389].

Squalene epoxidase (ERG1) and 2,3-oxidosqualene cyclase (ERG7) catalyze the synthesis of squalene epoxide and lanosterol, respectively. It was found that allylamines, naftifine, and terbinafine are reversible inhibitors of the Candida ERG1 (Table 5) [390,391]. In addition to allylamines, the thiocarbamates toliclate and tolnaftate were also shown to be potent inhibitors of ERG1 (Table 5) [392]. Table 5 clearly demonstrates that all presented allylamines and thiocarbamates are more effective against T. rubrum ERG1 than against C. albicans squalene epoxidase. However, the resistance of Trichophyton spp. to terbinafine, licensed for the treatment of dermatophytic infections, increases dramatically [393–395], creating a serious limitation to its further clinical application. Moreover, there is evidence in favor of terbinafine-induced hepatotoxicity [396]. The emerging resistance of dermatophytes to terbinafine and the moderate activity of both allylamines and thiocarbamates against C. albicans show the need for a further search for highly effective ERG1 inhibitors.

Lanosterol is converted to zymosterol via two intermediates, 4,4-dimethyl-zymosterol-8,14,24-trienol and 4,4-dimethyl-zymosterol, by lanosterol 14α-demethylase (ERG11) and sterol C14-reductase (ERG24). Azoles were identified as effective inhibitors of ERG11 (Table 5) via selective coordination with heme iron [397,398] and demonstrated striking antifungal activity against a variety of human fungal pathogens [399,400]. Importantly, azoles inhibit human lanosterol 14α-demethylase at substantially higher concentrations than the fungal enzyme [397]. A series of steroidal 1,4-dihydropyridines also showed promising activity against various Candida spp. via the inhibition of ERG11 [401]. Despite the pronounced activity of azoles against Candida spp. ERG11 (Table 5), the enlarged resistance to azoles by Candida species is a serious threat in their clinical use [402,403]. Therefore, the novelazole-based derivatives could attract attention as ERG11 inhibitors.

In the fungal cell, sterol C24-methyltransferase (ERG6) transforms zymosterol to fecosterol. It is converted to episterol by sterol C8,7-isomerase (ERG2). Sterol C5(6)-desaturase (ERG3) converts episterol to ergosta-5,7,24(28)-trienol [404–406]. NADPH-dependent sterol C22-desaturase (ERG5) catalyzes the formation of the next intermediate, ergosta-5,7,22,24(28)-tetraenol. At the final step, NADPH-dependent sterol C24-reductase (ERG4) converts ergosta-5,7,22,24(28)-tetraenol to ergosterol molecules [365]. It was demonstrated that amorolfine, fenpropidin, fenpropimorph, and the related morpholines and piperidines act as dual inhibitors of ERG24 and ERG2 [407,408]. Thus, these compounds seem to be ideal antifungals, as acquiring resistance against them will be difficult for pathogens because of the requirement to mutate two enzyme genes at once. Among the aminopiperidine derivatives, as presented in Table 5, compound 1b has a lower IC50 value, and the substantial prolongation of survival in infected mice with its oral administration [409] indicates the potential clinical benefits. However, presently, only amorolfine is used clinically to treat nail infections.

It should be noted that zymosterol is a precursor in cholesterol biosynthesis in mammalian cells [410] (this branch of the biosynthetic pathway is marked with an orange
color in Figure 7). Ergosta-5,7,24(28)-trienol is a precursor of phytosterols, campesterol, \( \beta \)-sitosterol, and stigmasterol [411] (this branch of the biosynthetic pathway is indicated with a green color in Figure 7).

**Table 5.** Major inhibitors of fungal sterol biosynthesis.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Structure</th>
<th>Enzyme</th>
<th>IC(_{50}), ( \mu \text{M} )</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>terbinafine</td>
<td><img src="image" alt="Terbinafine Structure" /></td>
<td>ERG1</td>
<td>C. albicans 0.03</td>
<td>[390,392]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C. parapsilosis 0.02–0.04</td>
<td>[390]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C. glabrata 0.137</td>
<td>[390]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Trichophyton rubrum 0.002–0.016</td>
<td>[390,392]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A. fumigatus 0.24</td>
<td>[390]</td>
</tr>
<tr>
<td>naftifine</td>
<td><img src="image" alt="Naftifine Structure" /></td>
<td>ERG1</td>
<td>C. albicans 1.1</td>
<td>[390]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C. parapsilosis 0.34</td>
<td>[390]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T. rubrum 0.115 ± 0.030</td>
<td>[392]</td>
</tr>
<tr>
<td>SDZ 87-469</td>
<td><img src="image" alt="SDZ 87-469 Structure" /></td>
<td>ERG1</td>
<td>T. rubrum 0.020 ± 0.005</td>
<td>[392]</td>
</tr>
</tbody>
</table>
### Table 6: Effect of Antifungal Lipopeptides and Polyene Macrolides on Fungal Permeability

<table>
<thead>
<tr>
<th>Compound</th>
<th>ERG</th>
<th>Target Organism</th>
<th>Minimum Inhibitory Concentration (MIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tolciclate</td>
<td>ERG1</td>
<td>C. albicans</td>
<td>0.011 [392]</td>
</tr>
<tr>
<td>tolnaftate</td>
<td>ERG1</td>
<td>T. rubrum</td>
<td>0.028 ± 0.003 [392]</td>
</tr>
<tr>
<td>bifonazole</td>
<td>ERG11</td>
<td>C. albicans</td>
<td>0.3 [397]</td>
</tr>
<tr>
<td>clotrimazole</td>
<td>ERG11</td>
<td>C. albicans</td>
<td>0.091 [397]</td>
</tr>
<tr>
<td>miconazole</td>
<td>ERG11</td>
<td>C. albicans</td>
<td>0.072 [397]</td>
</tr>
<tr>
<td>fluconazole</td>
<td>ERG11</td>
<td>C. albicans</td>
<td>0.051–0.6 [397,412]</td>
</tr>
<tr>
<td>fluconazole</td>
<td>ERG11</td>
<td>C. neoformans</td>
<td>0.17 [413]</td>
</tr>
<tr>
<td>fluconazole</td>
<td>ERG11</td>
<td>Malassezia globosa</td>
<td>0.206 ± 0.008 [414]</td>
</tr>
<tr>
<td>itraconazole</td>
<td>ERG11</td>
<td>C. albicans</td>
<td>0.039–0.4 [397,412]</td>
</tr>
<tr>
<td>itraconazole</td>
<td>ERG11</td>
<td>C. neoformans</td>
<td>0.17 [413]</td>
</tr>
<tr>
<td>itraconazole</td>
<td>ERG11</td>
<td>M. globosa</td>
<td>0.188 ± 0.008 [414]</td>
</tr>
<tr>
<td>voriconazole</td>
<td>ERG11</td>
<td>C. neoformans</td>
<td>0.17 [413]</td>
</tr>
<tr>
<td>VT-1129</td>
<td>ERG11</td>
<td>C. neoformans</td>
<td>0.16 [413]</td>
</tr>
<tr>
<td>ketoconazole</td>
<td>ERG11</td>
<td>C. albicans</td>
<td>0.064–0.5 [397,412]</td>
</tr>
<tr>
<td>ketoconazole</td>
<td>ERG11</td>
<td>M. globosa</td>
<td>0.176 ± 0.016 [414]</td>
</tr>
<tr>
<td>ketaminazole</td>
<td>ERG11</td>
<td>M. globosa</td>
<td>0.321 ± 0.042 [414]</td>
</tr>
<tr>
<td>compound 1a</td>
<td>ERG24</td>
<td>C. albicans</td>
<td>0.063 [415]</td>
</tr>
<tr>
<td>compound 1b</td>
<td>ERG24</td>
<td>C. albicans</td>
<td>0.016 [415]</td>
</tr>
</tbody>
</table>

### 3.2. Agents with Direct Action on Fungal Lipid Membrane

The principles of action of naturally occurring antibiotics on fungal membranes are similar to those of antibacterial peptides and lipopeptides (Figure 4).

Table 6 summarizes the data concerning the effect of antifungal lipopeptides and polyene macrolides on the permeability of lipid bilayers that mimic the cell membranes of target fungi. One of the most attractive groups is the cyclic lipopeptides, which are secondary metabolites of certain bacteria and are used to combat plant fungal pathogens. It is well known that the syringomycins and syringopeptines from *Pseudomonas syringae,*
and surfactins, fengycins, iturins, bacillomycins, and mycosubtilin from \textit{B. subtilis}, form the transmembrane pores in model lipid membranes [416–427].

Table 6. The effects of antifungal agents on the model lipid membrane’s permeability.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Structure</th>
<th>( C_{\text{min}}, \mu\text{M} )</th>
<th>( C_{\text{tr}}, \mu\text{M} )</th>
<th>Target Lipid</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pore formation</strong></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>syringomycin E</td>
<td>\begin{align*} \text{Phe} &amp; \text{-Arg} \text{-Dab} \text{-Dab} \text{-D-Ser} \text{-Ser} \text{-CO} \text{-CH}_2 \text{-CH} \text{-CH}_2 \text{-CH}_2 \text{-OH} \ &amp; \text{DPhPC} \end{align*}</td>
<td>1–5</td>
<td>–*</td>
<td>DPhPC; DOPS:DOPE 1:1 (m/m)</td>
<td>[422]</td>
</tr>
<tr>
<td>syringopeptin 22A</td>
<td>\begin{align*} \text{Val} &amp; \text{-Val} \text{-Dab} \text{-D-Ser} \text{-Thr} \text{-CO} \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-OH} \ &amp; \text{DPhPC} \end{align*}</td>
<td>0.003</td>
<td>–*</td>
<td>DOPS:DOPE 1:1 (m/m)</td>
<td>[416]</td>
</tr>
<tr>
<td>syringopeptin 25A</td>
<td>\begin{align*} \text{Val} &amp; \text{-Val} \text{-Dab} \text{-D-Ser} \text{-Thr} \text{-CO} \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-OH} \ &amp; \text{DPhPC} \end{align*}</td>
<td>0.004</td>
<td>–*</td>
<td>PC:PE:PS 2:2:1 (m/m)</td>
<td>[428]</td>
</tr>
<tr>
<td>fengycins</td>
<td>\begin{align*} \text{Asp} &amp; \text{-D-Lou} \text{-L-Glu} \text{-L-Tyr} \text{-D-Val} \text{-L-D-Ser} \text{-Ser} \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-OH} \ &amp; \text{POPC:POPE:POPG:e} \text{-} \text{rgosterol 2:2:5:1 (m/m)} \end{align*}</td>
<td>0.1–0.5</td>
<td>&gt;10</td>
<td></td>
<td>[425]</td>
</tr>
<tr>
<td>surfactin</td>
<td>\begin{align*} \text{Asp} &amp; \text{-D-Tyr} \text{-Amp} \text{-CO} \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-OH} \ &amp; \text{PC:PE} \text{-} \text{PS} 2:2:1 (m/m) \end{align*}</td>
<td>0.2–0.4</td>
<td>–*</td>
<td>DPhPC</td>
<td>[424]</td>
</tr>
<tr>
<td>iturin A</td>
<td>\begin{align*} \text{Asp} &amp; \text{-D-Tyr} \text{-Amp} \text{-CO} \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-OH} \ &amp; \text{PC:PE} \text{-} \text{PS} 2:2:1 (m/m) \end{align*}</td>
<td>1.4</td>
<td>–*</td>
<td>glyceryl monooleate</td>
<td>[426]</td>
</tr>
<tr>
<td>mycosubtilin</td>
<td>\begin{align*} \text{Asp} &amp; \text{-D-Tyr} \text{-Amp} \text{-CO} \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-OH} \ &amp; \text{PC:PE} \text{-} \text{PS} 2:2:1 (m/m) \end{align*}</td>
<td>0.001</td>
<td>–*</td>
<td>egg-PC; egg-PC:DMPE 8:2 (v/v)</td>
<td>[429]</td>
</tr>
<tr>
<td>bacillomycins</td>
<td>\begin{align*} \text{Asp} &amp; \text{-D-Tyr} \text{-Amp} \text{-CO} \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-OH} \ &amp; \text{PC:PE} \text{-} \text{PS} 2:2:1 (m/m) \end{align*}</td>
<td>–*</td>
<td>–*</td>
<td>glyceromonoolein</td>
<td>[421]</td>
</tr>
<tr>
<td>amphotericin B</td>
<td>\begin{align*} \text{Asp} &amp; \text{-D-Tyr} \text{-Amp} \text{-CO} \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-OH} \ &amp; \text{PC:PE} \text{-} \text{PS} 2:2:1 (m/m) \end{align*}</td>
<td>0.02–0.03</td>
<td>–*</td>
<td>phospholipid:cholesterol 20:1 (m/m)</td>
<td>[431]</td>
</tr>
<tr>
<td>nystatin</td>
<td>\begin{align*} \text{Asp} &amp; \text{-D-Tyr} \text{-Amp} \text{-CO} \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-OH} \ &amp; \text{PC:PE} \text{-} \text{PS} 2:2:1 (m/m) \end{align*}</td>
<td>0.01</td>
<td>&gt;20</td>
<td>DPhPC:ergosterol 2:1 (m/m)</td>
<td>[432]</td>
</tr>
<tr>
<td>filipin</td>
<td>\begin{align*} \text{Asp} &amp; \text{-D-Tyr} \text{-Amp} \text{-CO} \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-OH} \ &amp; \text{PC:PE} \text{-} \text{PS} 2:2:1 (m/m) \end{align*}</td>
<td>0.02</td>
<td>–*</td>
<td>phospholipid:cholesterol 2:1 (v/v)</td>
<td>[434]</td>
</tr>
<tr>
<td>piscidin</td>
<td>\begin{align*} \text{Asp} &amp; \text{-D-Tyr} \text{-Amp} \text{-CO} \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-CH}_2 \text{-OH} \ &amp; \text{PC:PE} \text{-} \text{PS} 2:2:1 (m/m) \end{align*}</td>
<td>0.01</td>
<td>&gt;100</td>
<td>DPhPC:ergosterol 2:1 (m/m)</td>
<td>[435]</td>
</tr>
</tbody>
</table>
Antibiotics

12, 1716

mutations in the clinical strains of amphotericin B through a decrease in ergosterol content makes resistant strains biosynthetic pathway (2.1.4). It should be noted that the emergence of resistance to deletion of inhibit result antibiotics, the microdomains, and its pore might suggest t catalyzed by and decrease in the length of fatty acid chains and sphingolipid content. Mutants were promoting membrane disruption [448].

A pore of 3, an antifungal polyene isolated from a marine dinoflagellate, is also able to induce sphingomyelin proteins, it penetrating the membrane [444]. In addition to natamycin[432 formation of transmembrane pores in the target amino acids: DMPE DOPG POPG POPE POPC DOPE DOPS Abbreviations: threshold concentration required to disintegrate the lipid bilayers; *—data are absent.

As expected for the sterol — mast cells of fish exert their fungicidal effects on Antimicrobial peptides also demonstrate antifungal efficiency. Piscidins identified in Another clinically important group is antifungal macrolides and polyene antibiotics. Amphotericin B, nystatin, and fillipin demonstrate antimicrobial activity via the formation of transmembrane pores in the target-sterol-containing membranes [432–434,438–443]. The three-dimensional structure of the amphotericin B channel was proposed as an asymmetric heptamer complex of polyene and sterol molecules penetrating the membrane [444]. In addition to natamycin’s inhibitory effect on transport proteins, it was suggested to specifically interact with the sterol- and sphingomyelin-enriched ordered phase and disrupt lipid packing [437,445]. Amphidinol 3, an antifungal polyene isolated from a marine dinoflagellate, is also able to induce pore-like defects in model membranes [446]. Antimicrobial peptides also demonstrate antifungal efficiency. Piscidins identified in the mast cells of fish exert their fungicidal effects on C. albicans by disrupting the fungal membranes through pore formation [436,447]. An antimicrobial peptide from the tree frog Hyla punctata, hylaseptin P1-NH₂, demonstrates strong antifungal potential by promoting membrane disruption [448].

Saccharomyces cerevisiae strains, resistant to syringomycin E, are characterized by a decrease in the length of fatty acid chains and sphingolipid content. Mutants were defective in two key enzymes of the terminal sphingolipid biosynthetic pathway, IPCS and IPS (2.1.3) [449]. The sensitivity of S. cerevisiae towards syringomycin E was also shown to depend on the C₃-hydroxylation of sphingoid bases to form phytoceramide, catalyzed by SCH [450]. The relevance of M(IP)₃C [451] and sphingolipid C₃-hydroxylation [452] for the lateral segregation of lipids in S. cerevisiae membranes might suggest that syringomycin E may interact with sphingolipid-enriched microdomains, and its pore-forming ability is sensitive to their composition [453,454].

As expected for the sterol-dependent mechanism of pore formation by polyene antibiotics, the decline in the ergosterol content in the plasma membranes of target fungi results in the development of resistance [455]. In fact, it was found that the minimal inhibitory concentration of amphotericin B against C. albicans was increased by the deletion of ERG2, ERG6, ERG3, and ERG11, the enzymes participating in the ergosterol biosynthetic pathway (2.1.4). It should be noted that the emergence of resistance to amphotericin B through a decrease in ergosterol content makes resistant strains extremely sensitive to osmotic and other types of stress. The reduced level of ergosterol in clinical strains of Candida lusitaniae, which is resistant to amphotericin B, might arise from mutations in the ERG3 gene [456].
4. Antivirals Targeting Lipid Envelope

Since we have narrowed our focus to reviewing only compounds that directly target pathogen membranes, antivirals that have been shown to affect the membranes of virions, which lead to the destruction of the lipid envelope or suppression of virus fusion with the host cell, are discussed below.

Many socially significant viruses are enveloped, i.e., the virions are surrounded by a supercapsid composed of a lipid bilayer. Despite the fact that the origin of the lipid envelope is the host cell membrane, in some cases, a quantitative difference has been found in the content of various lipids in the viral envelope and the host cell membrane from which the virions have been budded [457–461]. This may also be due to virus-induced changes in the host cell’s lipid metabolism [462,463]. Thus, the lipid membranes of enveloped viruses might be considered a target for innovative antiviral drugs. The compounds are thought to break the lipid envelopes of virions or dramatically change the properties of the viral membrane in order to prevent fusion with the cell membrane. A significant advantage of using such an approach is the broadening of the spectrum of antiviral activity and a decrease in the resistance to viral pathogens.

4.1. Disrupting Agents

4.1.1. Photosensitizing Antivirals

Photosensitizers are compounds that can absorb light and generate reactive oxygen species, which, in turn, leads to the peroxidation of membrane lipids and damage to the lipid bilayers of both viral and cellular membranes [464] (Figure 8A). In the absence of virus systems for reparation, the photodamage of the lipid envelope causes a dramatic reduction in the infectivity of virions due to the inactivation of viral fusion. Among the photosensitizers, compounds with absorption in the infrared region are of particular interest in the search for new broad-spectrum antivirals due to their substantially higher tissue transparency for the radiation of this spectrum [465].

*Hypericin*, a plant-occurring polycyclic quinone, demonstrated broad-spectrum activity against enveloped viruses such as human immunodeficiency virus type 1 (HIV-1), Moloney murine leukemia virus, equine infectious anemia virus, vesicular stomatitis virus (VSV), herpes simplex virus types 1 (HSV-1) and 2 (HSV-2), parainfluenza virus (PIV), vaccinia virus, murine cytomegalovirus (mCMV), and Sindbis virus (SINV) [466–471] (Table 7). *Hypericin* did not alter non-enveloped viruses [467]. Halogen derivatives of hypericin were shown to be effective against HSV-1 [472,473]. *Gymnochromes* isolated from *Gymnocrinus richer* were shown to be highly potent antiviral agents against dengue virus, HSV-1, and influenza virus type A (IVA) [474,475] (Table 7). *Hypocrellins* from *Hypocrella bambuase* also demonstrated light-dependent anti-HIV, anti-mCMV, anti-HSV-1, anti-VSV, and anti-IVA efficacy [469,476–478] (Table 7), while the non-enveloped virus was not inactivated [477].
Initially, rigid amphipathic perylene-containing nucleoside derivatives, particularly 5-(perylen-3-yl)ethynyl-2′-deoxy-uridine (dUY11) and 5-(perylen-3-yl)ethyl-arabino-uridine (aUY11), having considerable activity against IVA, hepatitis C (HCV), HSV-1, HSV-2, mCMV, VSV, SINV, tick-borne encephalitis virus (TBEV), yellow fever virus (YFV), Chikungunya virus (CHIKV), African swine fever virus, PIV, and human respiratory syncytial virus (RSV) (Table 7), were believed to inhibit viral fusion by affecting membrane curvature stress (Section 3.2) [479–481]; however, later, their action through the photosensitization of viruses was postulated [482–487]. No activity or the non-specific activity of perylene derivatives against non-enveloped viruses was found [480,488]. Non-nucleoside perylene derivatives also showed promising antiviral activity [489].

BODIPY-based photosensitizer 2,6-diiodo-1,3,5,7-tetramethyl-8-(N-methyl-4-pyridyl)-4,4′-difluoroboradiazaindacene (DIMPy-BODIPY) exhibited the photodynamic inactivation of dengue virus and VSV at nanomolar concentrations [490].

A class of thiazolidine-based lipophilic inhibitors of LJ and JL series demonstrating high activity against a variety of enveloped viruses (Table 7), with no effect on the infection of non-enveloped viruses, was also originally described as curvature-induced antivirals (Section 3.2), but it was later shown that the compounds act as membrane-targeted photosensitizers [491–493].

Cationic imidazoly1 and pyridyl porphyrins were characterized as promising photosensitizing antivirals against SARS-CoV-2 and HSV-1 [494,495]. A natural chlorine photosensitizer, phaeophorbid a, inactivates HSV-1, HSV-2, MERS-CoV, SARS-CoV-2, YFV, HCV, and SINV, by targeting the lipid envelope [496,497] (Table 7).

<table>
<thead>
<tr>
<th>Photosensitizer</th>
<th>Structure</th>
<th>Virus</th>
<th>IC$_{50}$, μM</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>hypericin</td>
<td><img src="image" alt="Hypericin" /></td>
<td>HIV-1</td>
<td>0.44</td>
<td>[466]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HSV-1</td>
<td>0.006</td>
<td>[469]</td>
</tr>
<tr>
<td>Gymnochrome B</td>
<td>Dengue</td>
<td>0.029</td>
<td>[475]</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>----------</td>
<td>--------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>Hypocrellin A</td>
<td>HSV-1</td>
<td>0.015</td>
<td>[469]</td>
<td></td>
</tr>
<tr>
<td>Hypocrellin B</td>
<td>HSV-1</td>
<td>0.025</td>
<td>[469]</td>
<td></td>
</tr>
<tr>
<td>5-(perylen-3-yl)ethynyl-2′-deoxy-uridine (dUY11)</td>
<td>IVA</td>
<td>0.097–2.7</td>
<td>[480,498]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HSV-1</td>
<td>0.048–0.131</td>
<td>[479]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HSV-2</td>
<td>0.031–0.055</td>
<td>[479,480]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HCV</td>
<td>0.183–0.187</td>
<td>[479,480]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mCMV</td>
<td>0.037 ± 0.016</td>
<td>[480]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SINV</td>
<td>0.006 ± 0.001</td>
<td>[480]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TBEV</td>
<td>0.024 ± 0.013</td>
<td>[483,499]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PIV</td>
<td>2.2 ± 0.5</td>
<td>[498]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RSV</td>
<td>1.8 ± 0.2</td>
<td>[498]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SARS-CoV-2</td>
<td>0.2564</td>
<td>[487]</td>
<td></td>
</tr>
<tr>
<td>5-(perylen-3-yl)ethynyl-arabino-uridine (aUY11)</td>
<td>IVA</td>
<td>0.078–5.2</td>
<td>[480,498]</td>
<td></td>
</tr>
<tr>
<td>(Z)-5-[(5-phenylfuran-2-yl)methylidene]-3-prop-2-enyl-2-sulfanylidene-1,3-thiazolidin-4-one (LJ-001)</td>
<td>HIV</td>
<td>0.133</td>
<td>[492]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Newcastle disease virus</td>
<td>0.095</td>
<td>[492]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ebola virus</td>
<td>0.9</td>
<td>[492]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IVA</td>
<td>0.026</td>
<td>[492]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nipah virus</td>
<td>0.048</td>
<td>[492]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hendra virus</td>
<td>0.018</td>
<td>[492]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rift valley fever virus</td>
<td>0.02</td>
<td>[492]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Semliki forest virus</td>
<td>0.537</td>
<td>[492]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HSV-1</td>
<td>0.02</td>
<td>[492]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>hCMV</td>
<td>0.13</td>
<td>[492]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VSV</td>
<td>0.298</td>
<td>[492]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIV</td>
<td>0.013</td>
<td>[492]</td>
<td></td>
</tr>
</tbody>
</table>
3-ethyl-5-[5-(2-methoxyphenyl)-furan-2-ylmethyl]oxazolidine-2,4-dithione (JL-103)

<table>
<thead>
<tr>
<th>Structure</th>
<th>Virus</th>
<th>IC₅₀, μM</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>5,15-bis(1,3-dimethylimidazol-2-yl)chlorin (ICH-Me2+)</td>
<td>Newcastle disease virus</td>
<td>0.004</td>
<td>[492]</td>
</tr>
<tr>
<td></td>
<td>Ebola virus</td>
<td>0.185</td>
<td>[492]</td>
</tr>
<tr>
<td></td>
<td>IVA</td>
<td>0.002</td>
<td>[492]</td>
</tr>
<tr>
<td></td>
<td>Nipah virus</td>
<td>0.004</td>
<td>[492]</td>
</tr>
<tr>
<td></td>
<td>Hendra virus</td>
<td>0.0005</td>
<td>[492]</td>
</tr>
<tr>
<td></td>
<td>Rift valley fever virus</td>
<td>0.003</td>
<td>[492]</td>
</tr>
<tr>
<td></td>
<td>Semliki forest virus</td>
<td>0.044</td>
<td>[492]</td>
</tr>
<tr>
<td></td>
<td>HSV-1</td>
<td>0.002</td>
<td>[492]</td>
</tr>
<tr>
<td></td>
<td>hCMV</td>
<td>0.004</td>
<td>[492]</td>
</tr>
<tr>
<td></td>
<td>VSV</td>
<td>0.011</td>
<td>[492]</td>
</tr>
<tr>
<td></td>
<td>SARS-CoV-2</td>
<td>0.12</td>
<td>[494]</td>
</tr>
<tr>
<td></td>
<td>SARS-CoV-2</td>
<td>0.18</td>
<td>[497]</td>
</tr>
<tr>
<td></td>
<td>MERS-CoV</td>
<td>0.18</td>
<td>[497]</td>
</tr>
</tbody>
</table>

IC₅₀ is determined at photoactivation.

4.1.2. Tweezers

Molecular tweezers are membrane-destabilizing agents that can disrupt the virus lipid envelope and can be used as broad-spectrum antivirals against influenza A virus, respiratory syncytial virus, human immunodeficiency virus, herpes simplex viruses, human cytomegalovirus, Ebola and Marburg viruses, SARS-CoV, SARS-CoV-2, MERS-CoV, and other enveloped viruses. These small molecules act as pincers that bind lipid head groups and disrupt lipid ordering and packing in the virus lipid envelope, which results in the virions being unable to infect the cells [500] (Figure 8B).

A basing compound, CLR01, was shown to inhibit HIV-1, Ebola, Zika, herpes simplex (HSV-1, HSV-2), measles, influenza virus, and SARS-CoV-2 infection by directly targeting the viral membrane [501–503] (Table 8). Its close analog, CLR05, also possessed broad-spectrum antiviral activity [500]. CLR01 and CLR05 did not reduce infection by the non-enveloped adenovirus and encephalomyocarditis virus [500]. The membrane-disrupting and, consequently, the antiviral activity of CLR01 might be substantially potentiated by the introduction of C₄, C₇, or aromatic radicals to each phosphate group [500,503] (Table 8).

Table 8. Molecular tweezers and their antiviral activity.

<table>
<thead>
<tr>
<th>Molecular Tweezer</th>
<th>Structure</th>
<th>Virus</th>
<th>IC₅₀, μM</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLR01</td>
<td>[Diagram]</td>
<td>HIV-1</td>
<td>13.7–20.1</td>
<td>[501]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ebola</td>
<td>25.8</td>
<td>[502]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zika</td>
<td>8.2</td>
<td>[502]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HSV-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HSV-2</td>
<td>19.3</td>
<td>[500]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>measles</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IVA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SARS-CoV-2</td>
<td>76.7</td>
<td>[503]</td>
</tr>
<tr>
<td>CLR05</td>
<td>[Diagram]</td>
<td>Zika</td>
<td>38.1</td>
<td>[500]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HSV-1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.1.3. Antimicrobial Peptides

Although many antimicrobial peptides, especially defensins and cathelicidins, have been shown to possess antiviral effects [273,504,505], the mechanisms of antiviral action are highly pleiotropic and involve more than simply a direct effect on the viral membrane. Regarding the focus of this section, bomidin, a naturally occurring antimicrobial peptide that is active against a variety of enveloped viruses, including SARS-CoV-2, HSV, dengue virus, and CHIKV, was supposed to disrupt the viral membrane [506]. Plantaricin NC8 αβ, a two-peptide bacteriocin produced by Lactobacillus plantarum strains, was shown to inhibit SARS-CoV-2, IVA, flaviviruses Langat and Kunjin, and HIV-1, via permeabilizing and destroying their envelopes [507] (Table 9). It was demonstrated that the anti-HIV activity of a cyclic peptide from plants, kalata B1, resulted from the disruption of the membranes of HIV particles due to their raft-like lipid density and enrichment with PE [508].
Table 9. Antimicrobial peptides with direct antiviral action through lipid envelope disruption.

<table>
<thead>
<tr>
<th>Antimicrobial Peptide</th>
<th>Structure</th>
<th>Virus</th>
<th>IC_{50}, μM</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-plantaricin NC8 α</td>
<td>Asp - Leu - Thr - Thr - Lys - Leu - Trp - Ser - Ser</td>
<td>SARS-CoV-2</td>
<td>~0.001</td>
<td>[507]</td>
</tr>
<tr>
<td></td>
<td>Trp - Gly - Tyr - Tyr - Leu - Gly - Lys - Lys - Ala</td>
<td>IVA</td>
<td>~0.1</td>
<td>[507]</td>
</tr>
<tr>
<td>kalata B1</td>
<td>Trp - Arg - Trp - Asp - Leu - Lys - His - Pro - Tyr - Val</td>
<td>HIV</td>
<td>~2 - 5</td>
<td>[508]</td>
</tr>
</tbody>
</table>

4.2. Fusion Inhibitors Affecting Membrane Fluidity and/or Curvature Stress

An essential step in the fusion of an enveloped virus with a cell is the fusion of their lipid membranes. It is believed that this occurs in several successive stages, one of which includes the assembly of the contiguous outer lipid leaflets of the membranes to constitute an intermediate stalk. The stalk is characterized by a negative spontaneous curvature, corresponding to this formation via the cone-shaped lipids of an inverted hexagonal phase (HII) [509,510]. The induction of positive curvature stress by putative antiviral agents is believed to prevent the generation of fusion intermediates of negative curvature (Figure 8C).

Lipophosphoglycan dramatically reduced the fusion of Sendai virus and IVA with host cells [511,512], while it raised the bilayer-to-HII-phase transition temperature of phosphatidylethanolamine, indicating the elevation of positive curvature stress by lipophosphoglycan [511].

Naturally occurring and synthetic lipopeptides appear to be the most promising candidates when taking into account their amphiphilicity and cone shape, which suggests the induction of positive curvature when incorporated into a lipid monolayer. A simple lipopeptide sequence, myr-WD, was shown to successfully combat IVA and murine coronavirus infections by modulating the membrane lipid packing and surface potential [513]. Surfactin, a cyclic lipopeptide from B. subtilis, was found to inhibit porcine epidemic diarrhea virus and transmissible gastroenteritis virus infections via affecting curvature stress [514]. The dependence of the efficiency of surfactins to inhibit VSV, HSV-1, and Semliki forest virus on the length of the hydrocarbon “tail” was in good agreement with their membrane targeting [515]. Recently, the ability of several lipopeptides to successfully inhibit SARS-CoV-2 fusion with Vero cells was shown [516] (Table 10). The most effective compounds were also characterized by their marked ability to increase the transition temperature of phosphatidylethanolamine from the lamellar to HII phase, i.e., induce a positive curvature stress [516]. Interestingly, simpler molecules, such as black pepper alkaloid piperine, show a similar ability to suppress SARS-CoV-2 infection [517].

Table 10. Fusion inhibitors affecting membrane curvature stress and their antiviral activity.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Structure</th>
<th>Virus</th>
<th>IC{50}, μM</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>aculeacin A</td>
<td>SARS-CoV-2</td>
<td>1.3 ± 0.3</td>
<td>[517]</td>
<td></td>
</tr>
</tbody>
</table>
5. Conclusions

(i) Due to principal differences in the organization of fatty acid synthase systems in bacteria and mammals, the specific inhibitors of bacterial key enzymes, especially the acetyl-CoA-carboxylase complex, various β-ketoacyl-ACP synthases, different NADPH-dependent reductases, β-hydroxyacyl-ACP dehydrases, and acyl-phosphate:glycerol-3-phosphate acyltransferase, are attractive targets for the development of low-toxicity antibacterials.

(ii) The pathway for the synthesis of the lipid fatty acid tails in fungi is similar to that in mammalian cells and, therefore, is not very promising in the search for potential antifungals.

(iii) The presence of a single fundamental pathway for the synthesis of the phospholipid heads in both prokaryotes and eukaryotes makes the majority of the involved enzymes poor targets for antibiotic therapy in bacterial and fungal infections.

(iv) Many enzymes of the lipopolysaccharide (Kdo2-lipid A) biosynthetic pathway in Gram-negative bacteria (UDP-N-acetylglicosamine acyltransferase, UDP-3-O-(R-3-hydroxyacyl)glucosamine N-acyltransferase, UDP-3-O-(R-3-hydroxyacyl)-N-acetylglicosamine deacetylase, and UDP-diacylglucosamine pyrophosphohydrolase) are identified as targets for antibiotic development.

(v) Sphingolipid biosynthetic pathways are conserved from yeast to humans, and the enzymes cannot serve as targets for low-toxicity antifungals. Some inhibitors of inositol-phosphoceramide synthase demonstrate promisingly low effective concentrations.

(vi) The most effective approach when targeting fungal lipid biosynthesis is to search for inhibitors of enzymes in the ergosterol pathway, especially squalene epoxidase, lanosterol 14α-demethylase, and sterol C14-reductase/sterol C8,7-isomerase.

(vii) A preference given to inhibitors that simultaneously act on two enzymes of the lipid biosynthetic pathway or the combination of inhibitors with agents directly affecting the pathogen membrane should reduce the risk of developing antibiotic resistance in pathogenic strains.

(viii) Natural antimicrobial agents exert their defensive activities via pathogen membrane disruption due to pore formation or the disordering of membrane lipids. Due to the
high efficiency of naturally occurring antimicrobial agents, their broad-spectrum antibacterial/antifungal/antiviral effect, and their low rate of resistance in pathogen strains, the use of antimicrobial peptides, lipopeptides, and polyenes is a good anti-infective therapeutic strategy.

(ix) The lipid envelope of viruses should be considered as a target for innovative antivirals, disrupting the membranes of virions or inducing curvature stress and inhibiting viral entry.

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