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Review

Targeting of the Gram-Negative Outer Membrane for Antibiotic Discovery and Potentiation

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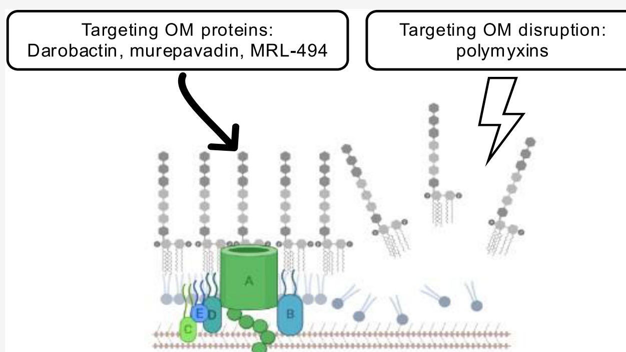

ABSTRACT: The emergence of multidrug resistance underscores the urgent need to develop new classes of antibiotics with novel mechanisms of action. The majority of antibiotics currently in use are designed to target Gram-positive bacteria. However, Gram-negative bacteria can circumvent the effects of the majority of drug molecules due to the unique composition of their outer membrane. This additional layer functions as a formidable barrier, impeding the penetration of compounds into the cell. In this context, several strategies have been identified to facilitate the accumulation of compounds in Gram-negative bacteria. This review provides a concise overview of the physicochemical properties that can assist in the entry and accumulation of compounds in Gram-negative bacteria, and it also covers various approaches for targeting or circumventing the outer membrane-mediated barrier of Gram-negative pathogenic bacteria.

KEYWORDS: *Gram-negative outer membrane, outer membrane biogenesis, β-barrel assembly machinery (BAM), lipopolysaccharide transport (Lpt), antibiotic potentiation, compound accumulation*

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INTRODUCTION

There is an urgent need to identify novel antimicrobials for multidrug-resistant Gram-negative pathogens.[1] Current treatment options inhibit only a limited number of essential bacterial processes and thus a limited number of intracellular targets. Therefore, the discovery of antimicrobials targeting other processes is crucial to avoid existing resistance mechanisms. Quinolones, the latest class of antibiotics active against Gram-negative bacteria, were introduced into the clinic in 1967. Since then, the antibiotic development pipeline has stalled, with no compound having entered clinical trials with a single mode of action.[2] This failure was largely due to the inability of small molecules to penetrate the bacterial envelope and reach their intracellular target(s) in sufficient quantity.[3,4]

Gram-negative bacteria are protected by two membrane barriers: the outer membrane (OM) and the inner membrane (IM).[5] The OM is an asymmetric bilayer composed of lipopolysaccharide (LPS) in the outer leaflet and phospholipids in the inner leaflet. The distinctive physical characteristics of the OM serve to create an essential permeability barrier that protects Gram-negative bacteria from environmental assaults. However, the OM barrier represents a significant obstacle to antimicrobial research. Conventional small-molecule screening efforts aimed at identifying active compounds against Gram-negative bacteria have largely been unsuccessful due to the incompatibility of their physical and chemical properties with the permeability barrier of the OM. Moreover, biochemical screens against key protein targets have yielded compounds that have proven difficult to optimize for both target potency and antibacterial activity. While the OM represents a significant challenge to discovering new antibiotics against Gram-negative bacteria, it also emerges as an attractive target for drug development. Indeed, disrupting the OM integrity or inhibiting the pathways involved in OM biogenesis results in the sensitization of bacteria to otherwise inactive compounds. Consequently, the OM can be directly targeted for developing novel antibiotics and offers a new prospect of enhancing the activity of existing antibiotics with potentiators. This review examines the potential for targeting Gram-negative OM via various approaches, including inhibiting the terminal biogenesis steps of its constituents, including that of OM proteins (OMPs), LPS, and lipoproteins, as well as chemical disruption.[6−10]

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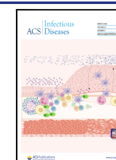

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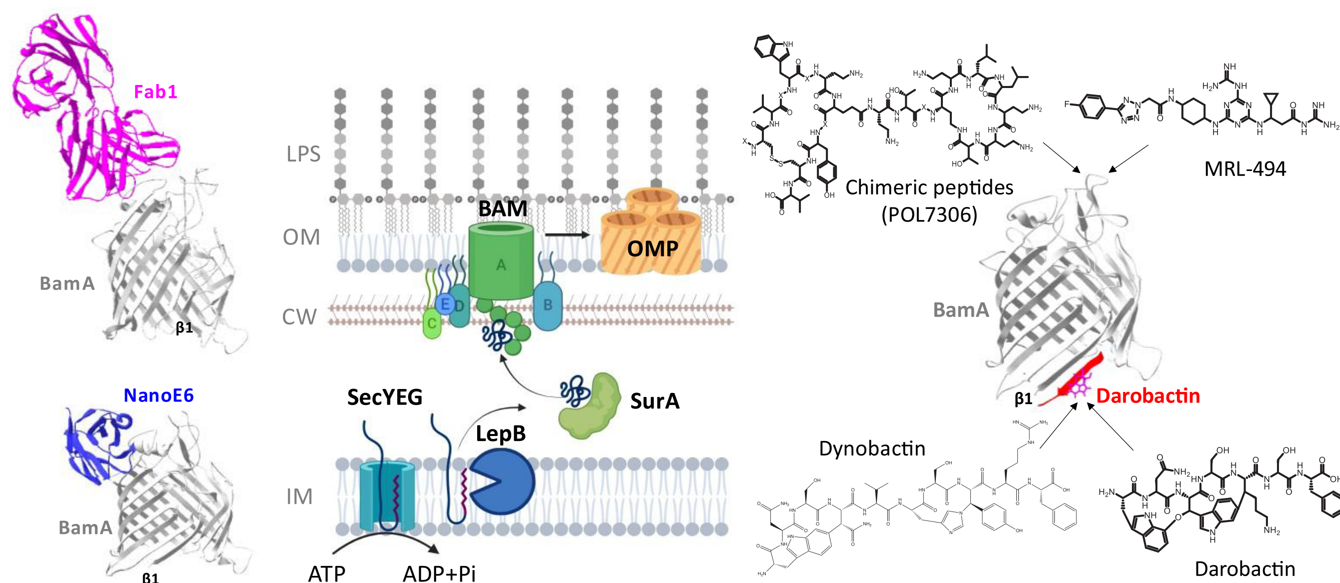

Figure 1. Targeting OMP biogenesis with BAM inhibitors. The central panel is a schematic representation of the Gram-negative cell envelope, illustrating the phospholipid inner membrane (IM), the periplasmic space containing the peptidoglycan cell wall (CW), and the outer membrane (OM) with phospholipids (PL) in the inner leaflet and lipopolysaccharide (LPS) in the outer leaflet. A comprehensive illustration of the essential stages of OMP biogenesis is presented. The illustration was created with BioRender.com. The lateral panels show structures of BamA that are bound by large and small molecules as follows: FAB1 (pink) is shown bound to extracellular loop 4 of BamA (gray) (PDB: 7ND0); NanoE6 (blue) is shown bound to extracellular loop 4 of BamA (gray) (PDB: 6QGW); darobactin (pink) is shown bound to the first β-strand (β1) of BamA (gray) at the periplasmic side (PDB: 7NRI). Dynobactin also likely interacts with β1 of BamA. In contrast, MRL-494 and chimeras between Murepavadin and colistin inhibit BamA from the extracellular side. Protein structures were generated with PDB Viewer.

Targeting OMP Biogenesis

The β-barrel assembly machinery (BAM) is responsible for properly folding and membrane insertion of OMPs[11] (Figure 1, central panel). Bam is a heteropentameric complex composed of the central component BamA and the four lipoproteins BamBCDE. In *Escherichia coli*, the C-terminal domain of BamA assembles as a β-barrel in the OM and the N-terminus folds into five Polypeptides Transport-Associated (POTRA) domains in the periplasm. BamA and BamD are the two essential proteins of the complex.[11,12] Before BAM takes over, nascent OMP polypeptides cross the IM via the Sec translocon, which recognizes their N-terminal signal sequence.[13] Once in the periplasm, chaperones (i.e., SurA or Skp/DegP) escort the nascent OMPs to the BAM complex.[14] The β-barrel of BamA is composed of 16 antiparallel β-strands, with the first and the last strands connected by a weak network of hydrogen bonds and forming a lateral gate. This dynamic lateral gate alternates between open and closed conformations critical for OMP folding and OM insertion.[15−17] In the gate, the β-signal at the C-terminus of the nascent OMP binds to the first β-strand of BamA.[18,19] The mechanism by which new OMPs are folded and transported through BamA is still under investigation. Nevertheless, as β-sheets are amphipathic, the β-barrel cannot fit the OM until completely closed. Two models of BamA operation have been proposed to aid the formation of such a cylinder: (i) in the budding model, the β-sheets of the nascent OMP fold by interacting with the BamA channel, forming a transient hybrid channel; (ii) in the assisted model, the β-barrel initiates folding within the periplasm by interacting with the BamA POTRA domains and then inserts into the OM via the BamA channel. While *in vivo* and *in vitro* experiments support the assisted model, the observation of BamA in a laterally open conformation favors the budding model.[20−24]

Proteins of the BAM complex, particularly BamA and BamD, are highly conserved among Gram-negative bacteria. The majority of OMPs are nonessential. However, defects in BamA or BamD that alter the function of the BAM complex are lethal due to the toxic accumulation of unfolded OMPs in the periplasm and the subsequent loss of OM integrity. The abundance of structural and biological data has motivated the search for BamA inhibitors. Moreover, as a surface-exposed protein, BamA is readily accessible from the extracellular environment, circumventing the issues associated with drug permeability and efflux, thus rendering it an appealing therapeutic target.

Several compounds have been identified that directly inhibit the BAM complex by interacting with BamA. Among these, the bis-guanidine MRL-494 was discovered in a screen for compounds that inhibit the growth of *E. coli* by disrupting the OM.[25] Mechanistic studies showed that MRL-494 causes a significant decrease in OMP levels and increases σ^E stress response activity. MRL-494 was also shown to inhibit the correct assembly and OM insertion of LamB, consistent with MRL-494 interfering with BAM-mediated OMP maturation.[25] Additional results indicated that MRL-494 interacts directly with BamA and prevents conformational changes necessary for OMP assembly. Notably, MRL-494 can also permeabilize the cytoplasmic membrane of Gram-positive bacteria, suggesting a second mode of action independent of BamA.[25] Chemical synthesis of analogs has allowed optimization for potency and selectivity against Gram-negative pathogens. Initial structure−activity analyses have shown that one of the two guanidine groups in this series is essential for antibacterial activity[26] (see Figure 1, right panel for the molecular structure of MRL-494).

Another small molecule, VUF15259, was identified in a screen for inhibition of autotransporter biogenesis.[27] This

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compound may act by inhibiting β-barrel folding, including that of BamA itself, although its target has not yet been determined.[27] However, mechanistic studies and compound optimization are needed to advance VUF15259 as a potential drug.

BamA and BamD are essential components of the BAM complex and interact directly in the periplasmic space through the fifth POTRA domain of BamA.[28] Therefore, chemical disruption of this interaction could prevent OMP folding and interfere with bacterial growth. This was exemplified by a peptide derived from the BamA C-terminus, which was found to interfere with BamA folding in an *in vitro* BAM reconstitution assay.[29] When the competing peptide was expressed and targeted to the periplasm of *E. coli*, growth defects and OM permeabilization were observed, consistent with the inhibition of the BAM complex formation.[29] Another peptide targeting the BamA-BamD interface was inspired by a conserved region of BamD that interacts with BamA in *Pseudomonas aeruginosa*.[30] This peptide had no antibacterial activity but potentiated the activities of several antibiotics, presumably by permeabilizing the OM.[30] Although these linear peptides lack drug-like properties and do not exhibit antibacterial activity, they highlight the potential druggability of the BAM complex assembly and provide new tools to explore the critical interface of BamA and BamD. Coupling this approach with structures of the BamA-BamD interface may enable the discovery of drug-like compounds. Indeed, Li et al. identified IMB-H4 as a disruptor of the BamA-BamD interaction in a small molecule library screen using a yeast two-hybrid approach.[31] Pull-down and biolayer interferometry assays indicated this disruption was due to the direct binding of IMB-H4 to BamA.[31] IMB-H4 shows moderate to weak activity against a panel of Gram-negative pathogens with minimum inhibitory concentrations (MICs) of 4 to 32 μg/mL.[31] In addition, IMB-H4 showed a synergistic effect with other antibiotics such as polymyxin B, vancomycin, and gentamicin against *E. coli*, probably due to its membrane-permeabilizing activity.[31] While these results are encouraging, optimizing this first hit by improving potency and reducing eukaryotic cytotoxicity is necessary to determine if this approach can lead to molecules with drug-like properties.

Given the surface-exposed nature of BamA, Storek et al. screened for monoclonal antibodies targeting the extracellular loops of BamA. They identified MAB1, which binds the extracellular loop L4 and shows bactericidal activity against *E. coli* Δ*waaD*, a mutant with truncated LPS.[32] MAB1 inhibits OMP biogenesis and disrupts OM integrity only when the LPS barrier is defective. Interestingly, spontaneous mutants resistant to MAB1 contained mutations in *lpxM*, which encodes a lipid A myristoyltransferase. The double mutant Δ*waaD*, Δ*lpxM* exhibited decreased membrane fluidity compared to the Δ*waaD* parental strain, suggesting that OM rigidity is associated with BAM function and resistance to MAB1.[32] Unfortunately, MAB1 lacks therapeutic potential due to a high frequency of resistant mutants ($>10^{-6}$) and activity limited to strains with abnormally high OM fluidity.[32,33] Other studies reported nanobodies binding to the extracellular loops of BamA. Nuclear magnetic resonance (NMR) spectroscopy and X-ray crystallography showed that nanoE6 binds to the extracellular loop L4 (Figure 1, left panel) and nanoF7 binds to L3 and L6 of BamA.[34] Furthermore, the structures indicated that these two nanobodies stabilize the lateral gate of BamA in an open and closed state, respectively. Unfortunately, antibacterial assays were not reported in this study.[34] However, it is hypothesized that these nanobodies block BAM function, leading to OM dysfunction and growth defects similar to MAB1, of which the antigen-binding fragment also locks BamA in an open-gate conformation[35] (Figure 1, left panel).

One of the most promising BamA inhibitors is probably darobactin, which was identified by Imai et al. in a screen of extracts from *Photorhabdus* isolates, which are symbionts of the gut microbiome of nematodes, against *E. coli*.[36] This natural product is a ribosomally synthesized and post-translationally modified peptide that exhibits potent bactericidal activity against a large panel of clinically relevant Gram-negative bacteria, including *E. coli*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, and *Acinetobacter baumannii*.[36] Darobactin is inactive against Gram-positive bacteria and has no toxigenic activity against human cell lines, thus demonstrating its high level of specificity.[36] Darobactin is a large molecule (965 Da) and therefore cannot cross the OM via *E. coli* porins (OmpF and OmpC) whose permeation cutoff is around 600 Da. Several lines of evidence from microscopy, transcriptome, and proteome analysis suggest that darobactin targets the OM. Resistant mutants isolated in the presence of darobactin at 4 times the MIC contained several mutations, but those mapping to *bamA* were sufficient to promote resistance.[36] A specific and direct interaction between darobactin and BamA was demonstrated by isothermal titration calorimetry, and NMR spectroscopy showed that darobactin most likely stabilizes BamA in a closed-gate conformation, preventing the exit of nascent OMPs to the OM.[36] Consistent with this, mutations conferring darobactin resistance were mapped to the lateral gate of BamA.[36] The mode of action of darobactin on BamA was then elucidated by cryo-EM and X-ray crystallography.[37] Darobactin adopts a β-strand conformation that mimics the native β-signal of OMPs and competes for binding at the BamA side gate, freezing the channel in its open-state conformation[37] (Figure 1, right panel). Notably, darobactin has demonstrated efficacy in mouse infection models, suggesting it may serve as a promising lead for further optimization.[38] Since the discovery of darobactin, several studies have focused on increasing the activity and production of darobactin.[38−41] Darobactin is a modified heptapeptide with unusual macrocycle cross-links. The biosynthetic gene cluster (BGC) responsible for darobactin production consists of *darA* encoding the propeptide, *darBCD* encoding an ABC transporter, and *darE* encoding a radical S-adenosylmethionine (SAM) enzyme. Groß et al. developed a heterologous expression system containing only *darA* and *darE*, which are essential for darobactin biosynthesis in *E. coli*, to enhance darobactin production and to design genetic variants.[39] This approach led to the identification of darobactin 9, a non-natural derivative that exhibited greater potency than darobactin A against both *P. aeruginosa* and *A. baumannii*.[39] In a separate study, Böhringer et al. examined multiple genomes within the Gammaproteobacteria clade to identify darobactin BGCs. Several darobactin orthologs (darobactin B−F) were identified and individually expressed in *E. coli* by modifying the original heterologous expression system.[40] This approach led to the identification of darobactin B.[40] More recently, a structural evaluation of the Bam complex with darobactin 9 was conducted using cryo-EM in conjunction with bioactivity evaluation.[41] This resulted in the biosynthetic engineering of novel derivatives with enhanced activity against Gram-negative pathogens, including clinically relevant isolates.

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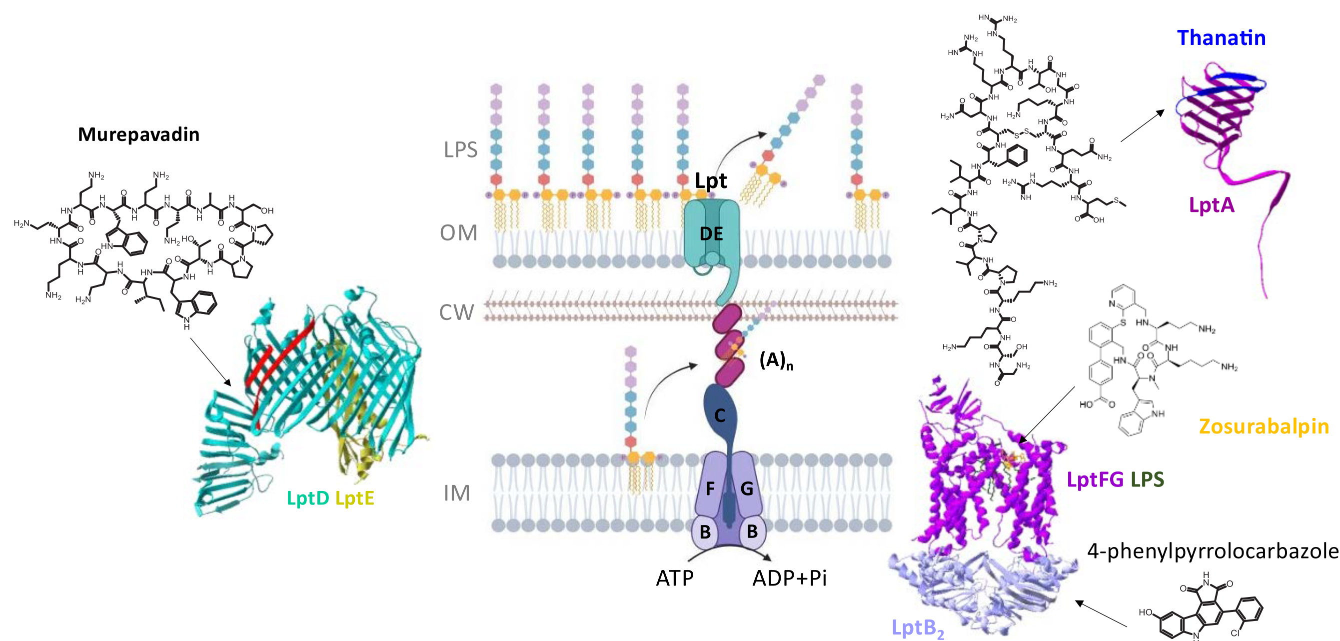

Figure 2. Targeting LPS biogenesis with Lpt inhibitors. The central panel is a schematic representation of the Gram-negative cell envelope, illustrating the phospholipid inner membrane (IM), the periplasmic space containing the peptidoglycan cell wall (CW), and the outer membrane (OM) with phospholipids (PL) in the inner leaflet and lipopolysaccharide (LPS) in the outer leaflet. A comprehensive illustration of the essential stages of LPS biogenesis is presented. The illustration was created with BioRender.com. The lateral panels show structures of proteins of the Lpt complex that are bound to inhibitors. Protein structures were generated with PDB Viewer using the PBD files 4q35 for the LptDE OM complex (in azure blue and gold), with β1 and β26 forming the lateral gate indicated in red; 6gd5 for the LptA monomer bound to thanatin (in bright pink and dark blue, respectively); 8frn for the LptB2FG inner membrane complex (in lilac and fuchsia) bound to LPS (in forest green) and zosurabalpin (in light orange).

The most promising derivative, darobactin 22, was verified through structure and activity-guided approaches, exhibiting up to 128-fold increased activity compared to natural darobactin A.[41]

Genome mining of distant BGCs enabled the identification of dynobactin A.[42] The results of antibacterial activity assays and structural analysis indicate that dynobactin A binds to the BamA lateral gate as previously observed for darobactins, despite structural differences.[42]

A series of chimeric peptides that combine β-hairpin derived from murepavadin and macrocycle derived from colistin was synthesized by Luther et al..[43] Murepavadin primarily targets the OMP LptD, which is involved in LPS transport (see below), while colistin targets the lipid A and disrupts the bacterial membranes. Derivative peptides **3**, **4**, **7**, and **8** exhibited potent and specific bactericidal activity against Gram-negative ESKAPE pathogens, including carbapenem- and colistin-resistant strains[43] (see Figure 1, right panel for the molecular structure of chimera **4**). Infection models in mice also demonstrated the efficacy of the compounds against these bacteria. Furthermore, their low toxicity to mammalian cells and low capacity to generate resistance make these peptides promising antibiotic candidates.[43] It is noteworthy that, rather than targeting LptD, photoaffinity and microscale thermophoresis experiments indicated that BamA was the most likely target. Specifically, NMR spectroscopy revealed an interaction with the outer L4, L6, and L7 loops of BamA, and it was observed that the BamA side gate was blocked in the closed state.[43] In addition, microscopic analyses revealed dramatic disruptions in membrane structure as well as permeabilization of both membranes in *E. coli*. Although these chimeras are particularly promising, this result raises the question of whether they are truly functioning by lethal blocking of the BAM complex or by permeabilizing the bacterial membranes.[43]

Despite compelling genetic and structural validation, BAM inhibition illustrates the challenges of translating OM targets into broadly effective therapeutics. Approaches that rely on macromolecular biologics, including monoclonal antibodies and nanobodies, are strongly constrained by OM composition, particularly LPS length, heterogeneity, and membrane fluidity, which vary substantially across species and clinical isolates.[4−6,32] In addition, extracellular loops of BamA exhibit significant primary sequence variability across species and strains, limiting the generalizability of loop-targeting agents that depend on conserved surface epitopes.[44,45] Consistent with these OM-level accessibility constraints, as exemplified by MAB1, antibacterial activity is often limited to strains with compromised LPS, and resistance can emerge at high frequency through alterations in lipid A acylation that increase membrane rigidity.[25,32] Small-molecule BAM inhibitors such as MRL-494 partially overcome these limitations but frequently exhibit secondary membrane-disruptive effects or residual cytotoxicity, complicating their therapeutic development.[25,26] In contrast, darobactins demonstrate that high-affinity, structure-guided engagement of a conserved and functionally constrained site—namely the BamA lateral gate—can yield potent and selective antibacterial activity with low resistance frequency and in vivo efficacy.[36−41] Collectively, these observations highlight that BAM is a viable antibacterial target only when inhibitors combine precise target engagement with compatibility across diverse OM architectures.

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Targeting LPS Transport

The OM is unique in that it is asymmetric and contains LPS in the outer leaflet. LPS is an amphipathic molecule. The hydrophobic portion, known as lipid A, is a bis-phosphorylated glucosamine disaccharide with four to seven acyl chains, depending on the bacterial species and growth conditions, which anchor LPS into the OM. The lipid A portion is covalently linked to the core oligosaccharide, which is composed of nonrepeating sugars with at least one ketodeoxyoctonoic acid (Kdo) as the first unit of the core. The O-antigen, composed of repeating units of one to six sugar residues, is the most distal, surface-exposed, and variable portion of the LPS.[46,47] Of note, *E. coli* K12 strains produce only the lipid A-core moiety, resulting in a rough (R) form of LPS.[48]

LPS is a vital component for most Gram-negative bacteria. Consequently, the pathways responsible for synthesizing and exporting LPS are present, conserved, and essential.

The ABC (ATP-binding cassette) transporter MsbA is the initial player in LPS transport across the IM, responsible for providing energy to the flipping of the lipid A-core.[49] Following MsbA-mediated flipping, the lipid A-core moiety is transferred to the periplasm, where it is decorated by the O-antigen residues.[50] Mature LPS is then extracted from the IM and transported through the periplasm to the cell surface by the LPS transport (Lpt) system, which is composed of seven proteins (LptA-G).[51] The unique architecture of this protein complex, which spans the entire envelope enables the simultaneous extraction of the lipid portion of the LPS from the hydrophobic environment of the IM and its protection from the aqueous environment during the trafficking across the periplasmic space; it then facilitates the transport of the bulky hydrophilic sugar portion of the LPS across the OM and prevents the insertion of fatty acid chains into the inner leaflet of the OM. At the IM, the $LptB_2FGC$ subcomplex is an unconventional ABC transporter that energizes the process. At the OM, the translocon formed by the β-barrel protein LptD and the lipoprotein LptE coordinates the final step of LPS assembly at the bacterial surface (Figure 2, central panel). The Lpt system functions as a single unit. Indeed, *E. coli* mutants depleted of any Lpt component show similar phenotypes with cell growth arrest, accumulation of abnormal membrane-like structures in the periplasm, and newly synthesized LPS atypically modified in the outer leaflet of the IM.[52]

Because of its importance in Gram-negative bacterial cell physiology, LPS biogenesis is considered a promising target for drug development. Potent inhibitors of lipid A biosynthesis have been identified in previous studies and are currently under development (e.g., LpxC inhibitors). In addition, considerable efforts have been made in recent years to elucidate the molecular mechanism of lipid A-core flipping by MsbA, and promising inhibitors have been identified so far.[53−56] However, these will not be discussed here. Finally, the growing understanding of the molecular mechanism underlying the LPS transport to the OM by the Lpt machinery has allowed the development of *in vitro* systems to specifically identify and elucidate the mode of action of inhibitors of the three critical steps in the process, namely the ATPase activity of LptB, the assembly of the LptAC bridge, and the release of LPS to the bacterial surface by LptDE.

LptD is the target of the most promising inhibitor of LPS transport. L27−11 is a 14-residue peptidomimetic that belongs to a family of macrocyclic hairpin-shaped cationic peptides derived from the membranolytic host defense peptide protegrin I (PG-I).[57,58] L27−11 is the result of iterative optimizations of a β-hairpin mimetic of PG-I with weak hemolytic activity and potent antimicrobial activity against *P. aeruginosa*.[57] Isolation of *P. aeruginosa* mutants resistant to POL7080 (a derivative of L27−11 with improved plasma half-life) and photoaffinity labeling studies identified LptD as the bacterial target of the peptide.[57] More recently, photolabeling coupled with mass spectrometry provided evidence that the peptide cross-links to the periplasmic segment of LptD, which contains a β-jellyroll domain and an N-terminal insert domain characteristic of *Pseudomonas spp*.[59] The binding of the peptide is expected to block LPS transport. As in *lptD* conditional mutants, exposure of wild-type bacteria to the peptide results in intracellular accumulation of membrane-like material, OM blebbing, and lipid A modifications (i.e., absence of PagL-mediated deacylation and PagP-mediated acylation together with an increase in ArnT-mediated addition of 4-amino-4-deoxy-L-arabinose), consistent with inhibition of LPS transport to the bacterial surface.[60]

The promising properties of POL7080, renamed Murepavadin (see Figure 2, left panel for the molecular structure of Murepavadin), permitted the peptide to complete Phase I and II clinical trials successfully. However, Phase III for patients with *P. aeruginosa* pneumonia had to be terminated due to a high incidence of kidney injury.[61] Currently, a Phase I clinical trial for an inhaled formulation of Murepavadin conducted by Spexis has been approved for treating *P. aeruginosa* infections in patients with cystic fibrosis.[62]

Thanatin was first identified in 1996 as an insect-derived antimicrobial peptide with broad-spectrum antibacterial and antifungal activity.[63] It is a 21-amino acid peptide whose C-terminal region adopts a β-hairpin structure in micelles, which is considered essential for antibacterial activity. Thanatin is bactericidal against *E. coli*, yet it does not induce membrane-permeabilizing effects. Despite the lack of knowledge regarding its mechanism of action, Vetterli et al. presented further evidence suggesting that thanatin inhibits LPS transport in *E. coli*.[64] Exposure of bacteria to thanatin induced intracellular accumulation of membrane material, typical of defective LPS transport.[64] Using photoaffinity labeling experiments, the authors identified LptD and LptA as the most likely targets of the peptide.[64] Accordingly, spontaneous mutants of *E. coli* were mapped in *lptA*.[64] The LptA-thanatin complex's tridimensional structure revealed that the peptide β-hairpin's N-terminal strand docks in parallel orientation to the first N-terminal β-strand in the β-jellyroll of LptA[64] (Figure 2, right panel). Interestingly, this region is involved in LptA dimerization and its interaction with LptC. The hypothesis that thanatin targets the bridge formation was confirmed by using a bacterial two-hybrid *in vivo* assay and surface plasmon resonance (SPR) experiments *in vitro*.[65,66] Thanatin is not a suitable drug candidate for further development due to its weak drug-like properties and rapid emergence of resistance.[64] However, new synthetic macrocyclic peptides have been discovered, exhibiting potent antimicrobial activity against enterobacteria and murine infection models, favorable absorption, distribution, metabolism, and excretion (ADME), and a good safety profile.[67] Furthermore, these lead candidates bind in the low nanomolar range to LptA and a mutant of LptA that confers resistance to thanatin. These observations validate the Lpt protein bridge as a viable antibiotic target to combat bacterial resistance.[67] However, it can be argued that

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these molecules are inactive against other pathogens, such as *A. baumannii*, due to the low sequence identity of the target LptA.[68]

Another molecule targeting the LptC-LptA interaction is the quinoline derivative IMB-881, which was discovered in a screen using a yeast two-hybrid system.[69] IMB-881 showed antibacterial activity against *E. coli*, and SPR experiments further identified LptA as a binding target for IMB-881, which suggests that it may prevent the oligomerization of LptA as well as the Lpt bridge formation.[69] More recently, an approach based on native mass spectrometry was developed to identify new inhibitors of LPS transport by disrupting protein−protein interactions within the Lpt bridge.[70] This study was performed on LptH, the *P. aeruginosa* orthologue of LptA, and showed that both thanatin and IMB-881 are potent inhibitors of LptH dimerization.[70] Promisingly, the chemical synthesis of quinoline derivatives combined with quantitative measurement of the LptH monomer/dimer equilibrium has identified the molecular determinants that best disrupt LptH dimerization.[70]

The ATPase activity of the soluble component LptB was employed as a means to identify inhibitors targeting the $LptB_2FGC$ subcomplex. The original system developed by Gronenberg & Kahne consisted of an NADH-coupled ATPase assay to monitor ATP hydrolysis by measuring the decrease in fluorescence intensity of NADH to screen a library of kinase inhibitors.[71] This system led to the initial identification of compounds belonging to two different structural classes. Analogs of the most promising of these inhibitors (**1a**, a 4-phenylpyrrolocarbazole) were synthesized and tested for ATPase activity of LptB or $LptB_2FGC$ using the detection of inorganic phosphate.[72] However, the inhibitors were much less active on the $LptB_2FGC$ complex than on LptB alone, suggesting that interactions between the individual components of the complex promote a stable and active conformation of LptB.[72] More importantly, the hydrophobic nature of these compounds restricts their activity only on "leaky" *E. coli* strains with a defective OM, thus limiting their druggability.[72]

More recently, macrocyclic peptides (MCPs) have been identified as a new class of antibiotics that target the LPS transport machinery.[73] This new class of small-molecule antibiotics was identified through a whole-cell phenotypic screening of an MCP library against a collection of bacterial pathogens. Among these, RO7075573 was found to have potent and specific activity against *A. baumannii*, including antibiotic-susceptible and carbapenem-resistant (CRAB) strains, with MICs ranging from ≤0.06 to 5 μg/mL, but was inactive against Gram-positive and other Gram-negative species.[73] This suggests that the compound interacts with a novel target compared to standard antibiotics. The clinical candidate, zosurabalpin (RG6006), is a second-generation zwitterionic MCP that showed potent activity against CRAB and improved tolerability. *In vivo*, the antibacterial effect of zosurabalpin was tested and confirmed in mouse models infected with CRAB.[73] Zosurabalpin is currently undergoing clinical trials conducted by Roche.

To identify the target of zosurabalpin, resistance studies and biochemical assays were conducted. Spontaneous mutations were first identified in genes encoding the LPS transporter, in particular in *lptFG*.[73] Then, assays monitoring LPS release from the $LptB_2FGC$ complex reconstituted in proteoliposomes revealed that zosurabalpin blocked LPS extraction from the complex. However, the $LptB_2FGC$ complex containing mutated LptF can suppress zosurabalpin activity and restore LPS transport.[73] The molecular mechanism by which zosurabalpin (compound **2**) and its analogs (**1**, RO7196472, and **3**, RO7075573) inhibit LPS transport was further investigated by cryo-EM with the *Acinetobacter baylyi* proteins.[74] The high-resolution structures showed that these compounds trap a substrate-bound conformation of the $LptB_2FG$ transporter, effectively stalling the system (Figure 2, right panel). The structures also revealed that the macrocyclic peptides bind only to the most conserved regions of the LPS lipid A-core.[74] This suggests that the variance of LPS structure alone could not explain the species selectivity of these drugs. Instead, it is most likely that differences in LptFG proteins are responsible for drug recognition.[74]

Although the Lpt pathway is genetically conserved and essential in many Gram-negative bacteria, its translational exploitation as an antibacterial target is constrained by pronounced species-specific differences in LPS biology and OM architecture.[5,8] In particular, the dispensability of LPS for viability in *Acinetobacter* species challenges the assumption that inhibition of LPS transport will universally result in bactericidal activity.[75] Instead, multiple studies indicate that genes involved in LPS biosynthesis and transport are conditionally essential, such that growth inhibition arises primarily from the toxic intracellular accumulation of LPS intermediates when upstream biosynthetic steps remain active.[75] This mechanism complicates efforts to broaden antibacterial activity across diverse Gram-negative pathogens and raises important considerations for resistance, as resistance could arise through loss or downregulation of LPS biosynthesis, thereby preventing the toxic accumulation required for antibacterial activity.[75] Clinical experience with murepavadin further highlights that potent target engagement does not guarantee systemic tolerability, underscoring the importance of modality choice, route of administration, and tissue exposure in mitigating toxicity.[61] Collectively, these observations indicate that LPS transport inhibitors are most likely to succeed when developed as species-tailored agents or as components of rational combination strategies, rather than as broadly acting monotherapies.[73,74]

Targeting Lipoprotein Transport

Except for a few proteins, OMPs can be divided into lipoproteins and β-barrel proteins.[5] The N-terminal cysteine of bacterial lipoproteins is covalently modified with an acyl group.[76] In *E. coli*, most lipoproteins are anchored to the inner leaflet of the OM through their lipid moiety, thereby exposing the bulk of the protein in the periplasm.[76] Lipoproteins perform a multitude of functions, including the formation and maintenance of cellular shape, the biogenesis of the OM, molecular transport, signal transduction, and bacterial motility.[76] Braun's lipoprotein, also designated Lpp, was the first lipoprotein to be identified and is the most abundant in *E. coli*.[77] Lpp is anchored to the inner leaflet of the OM and links the OM covalently to the peptidoglycan, thereby stabilizing the bacterial cell wall.

Lipoproteins are synthesized as precursors in the cytoplasm with an N-terminal signal sequence for translocation across the IM by the Sec or Tat translocon.[76] The C-terminus of the signal sequence contains a four-residue motif designated the lipobox, which is defined as [LVI][ASTVI][GAS]C.[78] The cysteine at the +1 position is the only truly conserved residue of the motif and is the target of acylation during lipoprotein maturation.[76] In the periplasm, lipoproteins are anchored to

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the IM by their signal sequence and processed by the activity of three consecutive enzymes located in the IM. First, the preprolipoprotein diacylglycerol transferase Lgt adds a diacylglycerol to the thiol group of the conserved cysteine residue.[76] Then LspA, a lipoprotein signal peptidase also designated signal peptidase II (SPaseII), cleaves the signal sequence, generating a diacylated apolipoprotein with the cysteine as the new N-terminal amino acid.[76] Finally, the apolipoprotein N-acyl transferase Lnt adds a third acyl group to the amino group of the cysteine residue.[76] In the case of lipoproteins destined for the inner leaflet of the OM, such as Lpp, the mature triacylated lipoprotein is exported to the OM via the localization of lipoproteins (Lol) pathway[76] (Figure 3).

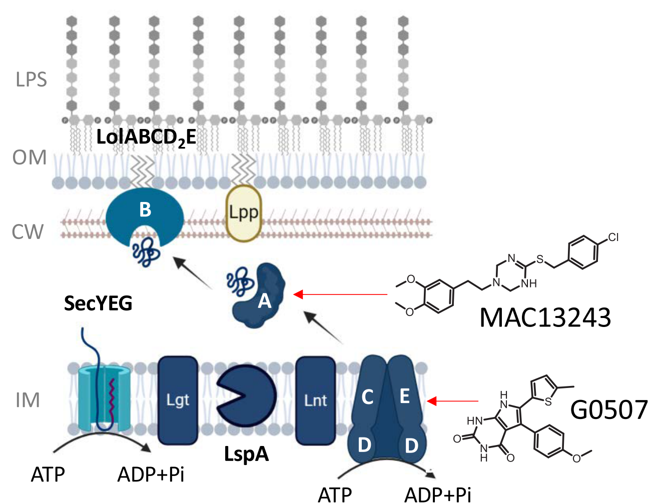

Figure 3. Targeting lipoprotein biogenesis with Lol inhibitors. Schematic representation of the Gram-negative cell envelope, illustrating the phospholipid inner membrane (IM), the periplasmic space containing the peptidoglycan cell wall (CW), and the outer membrane (OM) with phospholipids (PL) in the inner leaflet and lipopolysaccharide (LPS) in the outer leaflet. A comprehensive illustration of the essential stages of lipoprotein biogenesis is presented. The figure was created with BioRender.com.

The Lol system is composed of the ABC transporter LolCDE, the periplasmic chaperone LolA, and the OM lipoprotein receptor LolB. LolCDE is responsible for the energy-dependent extraction of lipoproteins from the IM and their transfer to LolA.[79,80] The lipoproteins are then trafficked across the periplasm, where they are passed to LolB, which catalyzes their insertion into the OM.[81] LolA and LolB share structural homology, both being incomplete β-barrels with a central hydrophobic cavity closed by an α-helical lid, but perform distinct functions.[82] LolB does not interact with LolC due, at least in part, to the absence of a C-terminal extension that in LolA binds a trio of surface residues of LolC.[83] Conversely, LolA is unable to release lipoproteins into membranes.[84,85] The precise mechanism of lipoprotein transfer between LolA and LolB remains unclear. However, in vivo cross-linking and NMR data suggest that this process occurs within the two cavities of the proteins, with the lipoproteins moving in a “mouth-to-mouth” model.[81,86]

Several lipoproteins involved in the biogenesis of the OM constituents are essential for bacterial growth: for example, LptE is a component of the LptDE translocon, while BamD is part of the Bam complex. Consequently, lipoprotein biogenesis represents an attractive target for drug discovery and development. Globomycin is a representative inhibitor of the lipoprotein maturation step, which targets LpsA.[87] The toxicity of globomycin and related compounds is the formation of cross-links between mislocalized Lpp and peptidoglycan.[88] Indeed, null mutations of *lpp* have been shown to relieve the toxicity of globomycin, although the lack of Lpp affects the integrity of the OM.[89] Recent advances in structural and biochemical data on lipoprotein-processing enzymes have provided new insights into the development of antimicrobials against Lgt and LpsA.[90−94] However, these compounds will not be discussed here.

Several compounds that inhibit the Lol pathway have been identified. CCT-00431 and CCT-00432 were the first Lol inhibitors discovered using a screening method that combined three assays.[95] Later, the compound MAC13243 was identified as a novel growth inhibitor of *E. coli* whose activity was suppressed by the overexpression of LolA[96] (see Figure 3 for the molecular structure of MAC13243). MAC13243 blocks lipoprotein targeting and inhibits LolA function through direct interaction, as shown by NMR. The MICs of MAC13243 against *E. coli* and *P. aeruginosa* are relatively high at 16 and 8 μg/mL, respectively. Nevertheless, it is encouraging that MAC13243 is poorly susceptible to efflux in *E. coli*, and remains lethal to numerous highly clinical isolates of *P. aeruginosa* while exhibiting low cytotoxicity toward eukaryotic cells.[96] Additionally, MAC13243 was identified in a screen for small molecules permeabilizing the OM of *E. coli*. In this study, MAC13243 was found to sensitize *E. coli* to large-scaffold antibiotics.[97] The question of whether this series is suitable for clinical use remains unanswered.

LolB is an essential lipoprotein. Only one study has reported inhibitors of the LolB protein produced by *Vibrio parahemolyticus* using high-throughput virtual screening.[98] The antibacterial effects of the compounds against *V. parahemolyticus* were shown to be concentration-dependent, with relatively high concentrations required for good inhibitory effects.[98] Fluorescence spectroscopy and molecular docking *in vitro* assays confirmed their binding affinity with LolB. In more detail, the inhibitors interact with LolB at the entrance of its hydrophobic cavity, where LolB receives the lipoprotein substrate from LolA.[81,98] However, further optimization of the compounds is necessary to enhance their antibacterial effects at lower concentrations.

Several inhibitors of the LolCDE subcomplex have been identified. In 2015, AstraZeneca conducted two screening campaigns to identify novel antibacterial drugs. High-throughput phenotypic screens were based either on an AmpC reporter system expressed in *E. coli* as a sensor of cell wall defects or the inhibition of *E. coli* growth. These screens led to the discovery of pyrazole and pyridineimadazole compounds, respectively.[99,100] Both compounds induce morphological changes analogous to those observed with globomycin, resulting in cell swelling, reduced nucleoid condensation, and increased membrane permeability.[99,100] Additionally, these compounds were demonstrated to inhibit the LolA-dependent release of Lpp from *E. coli* spheroplasts, and resistance-mapping mutations in LolC or LolE were identified.[99,100] While the target is promising, these compounds require optimization in numerous ways. Currently, the low solubility and high protein binding of these compounds are not adapted for dosing in mammals. The antibacterial activity of these compounds against *E. coli* is only moderate,

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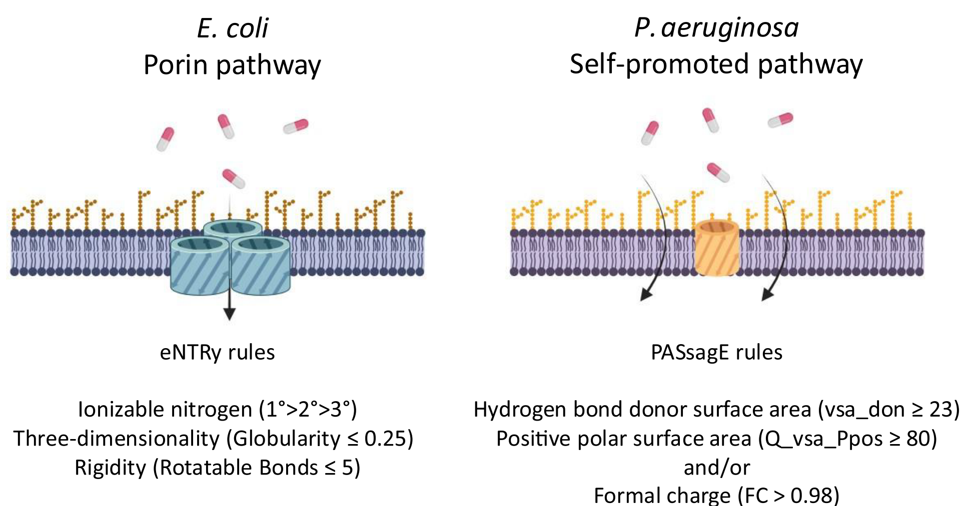

Figure 4. Differences in OM physiology and permeation guidelines for Gram-negative bacteria *E. coli* and *P. aeruginosa*. It has been demonstrated that compounds that comply with the eNTRY rule can enter *E. coli* through porins, while compounds following the PASsage rule can gain access via the self-promoted uptake pathway in *P. aeruginosa*. The figure was created with BioRender.com.

probably due to their high sensitivity to efflux and their limited spectrum of activity. They have no activity against other Gram-negative bacteria, such as *P. aeruginosa*, due to their inability to cross the OM. Other limiting features are the high frequency of resistance ($\sim 10^{-6}$) and the bacterial growth rebound during time-kill experiments.[99,100]

More recently, Genentech identified a pyrrolopyrimidinedione compound (G0507) in a phenotypic screen of *E. coli* growth inhibition followed by the selection of compounds inducing the σ^E stress response, which indicates OM perturbation[101] (see Figure 3 for the molecular structure of G0507). However, G0507 shares the same characteristics as those described above.

Targeting Molecular Rules of OM Permeation

A crucial aspect of developing a drug targeting Gram-negative bacteria is to focus on the enhanced accumulation of the compound within the bacterial cell. As such, several studies have been dedicated to investigating the relationship between the physicochemical properties of compounds and their accumulation in Gram-negative bacteria. The initial correlation between the physicochemical properties of a drug and its oral bioavailability was proposed by Lipinski et al.[102] They set five general rules as druggability guidelines for New Molecular Entities (NMEs) based on high-throughput screening (HTS) results. Following these guidelines, the bioavailability of a drug molecule is contingent upon pivotal physicochemical characteristics, including molecular weight, lipophilicity, and the number of hydrogen bonds. The molecule should possess no more than five hydrogen bond donors, no more than 10 hydrogen bond acceptors, a molecular mass of less than 500 Da, and a calculated water/*n*-octanol partition coefficient ($c \log P$) of less than 5. While the rule applies to numerous drugs, a considerable number of antibiotics do not adhere to the "rule of 5". In a separate study, O'Shea and colleagues analyzed a set of 147 active antibacterials and reported that antibacterial compounds occupy a distinct property space as compared to other therapeutic drug classes, exhibiting comparatively high molecular weight and low lipophilicity. A further analysis of the antibacterial properties revealed that compounds targeting Gram-positive and Gram-negative bacteria exhibit distinct physicochemical properties. The higher polarity of compounds targeting Gram-negative bacteria is a consequence of the compounds seeking their way through hydrophilic porin channels. Consequently, increased polarity and reduced size facilitate the entry of a compound into Gram-negative bacteria.[103] While these have been identified as crucial parameters for enhancing the penetration of drugs into Gram-negative bacteria OM via porins, these parameters alone are insufficient for the development of a broad-spectrum antibacterial drug. Furthermore, the diverse compositions of LPS and OM proteins among different bacterial species necessitate a more comprehensive investigation.

In this context, Hergenrother et al. conducted a mass spectrometry study of the accumulation of over 180 compounds (including approximately 70 Gram-positive-only antibiotics) in *E. coli* to identify common physicochemical properties of the accumulated compounds.[104] Based on their observations, general rules of accumulation were proposed, namely the presence of ionizable nitrogen, low three-dimensionality, and rigidity, herein referred to as eNTRY rules (Figure 4, left panel). In particular, it was observed that a positive charge was accompanied by enhanced accumulation. A closer examination revealed that primary amines play an important role in compound accumulation compared to secondary, tertiary, or quaternary amines. However, the primary amine is not the sole factor influencing accumulation, as evidenced by the observation that numerous compounds containing primary amines exhibit poor accumulation properties. Consequently, other essential factors were investigated across 297 molecular descriptors via computational analysis. Additionally, the rigidity (indicated by the number of rotatable bonds ≤ 5) and low three-dimensionality (quantified by a calculated globularity score ≤ 5) of the compounds appear to facilitate their accumulation.[104] Since then, guidelines for compound accumulation in *E. coli* have been used to rationalize antibiotic production.[105−108]

A similar methodology was used to examine the accumulation of compounds in *P. aeruginosa*. Following the experimental measurement of individual accumulation values for 345 compounds, the data obtained revealed certain characteristics associated with a positive charge and the number of hydrogen-donating bonds. Specifically, compounds

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with a suitable positive polar surface area (Q_VSA_PPOS ≥ 80) and/or a positive formal charge (FC ≥ 0.98), as well as a sufficient hydrogen bond donor surface area (vsa_don ≥ 23) have been demonstrated to have a high probability of accumulating in this species (Figure 4, right panel).[109] These rules, which we refer to here as PASsagE (*P. aeruginosa* Self-promoted Entry), compared to eNTRY, are not intended to be universally applicable. However, the analysis is of particular interest. *E. coli* eNTRY rules define permeation through porins as a positive charge-dependent process, whereas rigidity and low three-dimensionality impede efflux. In *P. aeruginosa*, the absence of the 40 porins typically expressed in this species does not affect the accumulation or activity of the aforementioned compounds.[109,110] Instead of using the porin pathway, the compounds find their way across the OM using the so-called self-promoted pathway, provided that the requisite PASsagE rules are satisfied. It is straightforward to envisage how a chimeric compound, comprising a nonpermeant antibiotic and a molecule that disrupts the OM, could accumulate and become active against *P. aeruginosa*. In this study, Hergenrother et al. confirmed this hypothesis by using a chimera between fusidic acid, a Gram-positive-only antibiotic unable to accumulate in *P. aeruginosa*, and a polyamine.[109] In retrospect, these observations demonstrate how molecular determinants provided by membrane permeabilizers enhance the accumulation and synergize the antibacterial activity of antibiotics that are unable to cross the OM of the most impermeable Gram-negative pathogens.

Interplay between OM Permeation, Efflux, and Antibiotic Potentiation

OM permeation and efflux act as tightly coupled determinants of intracellular drug accumulation in Gram-negative bacteria and should therefore be considered jointly during antibacterial design.[4,104] While permeation rules such as eNTRY and PASsagE identify physicochemical features that promote entry across the OM, they do not inherently address active efflux, which can negate accumulation even when permeation is efficient.[104,109] Several OM-targeting strategies described in this review, including inhibition of envelope biogenesis and the use of membrane-active potentiators, indirectly overcome efflux by increasing intracellular residence time rather than by directly inhibiting efflux pumps.[9,115] Importantly, many of these approaches operate without causing catastrophic membrane lysis, suggesting that nonlytic perturbation of OM integrity may provide a therapeutic window that enhances antibiotic activity while limiting host toxicity.[116−119] From this perspective, combination strategies—whether through coadministration of potentiators or rational molecular hybridization—should be viewed not as ancillary solutions, but as a central paradigm for overcoming intrinsic resistance in Gram-negative pathogens.[9,109]

Targeting OM Disruption

Polymyxins (polymyxin B (PMB) and polymyxin E (colistin)) are cyclic lipopeptide antibiotics with a high basicity resulting from the presence of five free primary amine groups. They are highly effective against Gram-negative bacteria, including the majority of *Enterobacteriaceae*, as well as *A. baumannii* and *P. aeruginosa*. All members of the polymyxin class contain a cyclic heptapeptide core, linked to a linear tripeptide with an N-terminal fatty acyl moiety.[111] The clinical use of polymyxins was discontinued in the 1960s due to nephrotoxicity. However, the emergence of highly drug-resistant Gram-negative bacterial strains has led to a resurgence of interest in polymyxins as a last-line treatment option. Nevertheless, the emergence of mobile colistin resistance (*mcr*) genes has prompted considerable concern and attention.[112] In addition, strains exhibiting resistance to polymyxins through alternative mechanisms, such as the decoration of the lipid A component of LPS by phosphorylethanolamine and 4-amino-arabinose, display high-level resistance and are a significant cause for concern.[113,114]

Polymyxins act specifically on Gram-negative bacteria and are rapidly bactericidal, whereas Gram-positive bacteria, eukaryotic microbes, and mammalian cells are typically unaffected. Polymyxins interact with anionic LPS molecules, which are exclusively present in Gram-negative bacteria and located in the outer leaflet of their OM, and induce OM damage. Although this mechanism has been extensively studied and well-characterized, the precise manner in which polymyxins interact with the IM and induce cell death remained unclear until recently. Notably, Sabnis et al. established that colistin resistance mediated by *mcr-1*, responsible for lipid A modification at the periplasmic side of the IM, protected the IM but not the OM from colistin-induced perturbations, indicating that colistin also targets LPS molecules in the IM during their transport to the OM.[115] Additionally, this study demonstrates that Murepavadin, which has been previously characterized as an inhibitor of LPS transport, renders *P. aeruginosa* more susceptible to colistin by increasing the abundance of LPS within the IM.[115] Some polymyxins, such as NAB741/SPR741, lack bactericidal activity but still damage the OM and impair its function as a permeability barrier to a range of noxious agents, including antibiotics.[116−119] Consequently, they act as "permeabilizers", "sensitizers", or "potentiators" at subinhibitory concentrations. For example, SPR741 has been demonstrated to exhibit robust synergistic activity with rifampicin and clarithromycin against a range of bacterial species, including *E. coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *A. baumannii*. As shown by Corbett et al., of the 35 antibiotics tested, the minimum inhibitory concentrations (MICs) of eight were reduced by 32 to 8,000-fold against *E. coli* and *K. pneumoniae* in the presence of SPR741. These included rifampicin, macrolides, and Gram-positive-only antibiotics such as fusidic acid.[116]

In recent years, a considerable amount of public funding has been allocated to the development of novel polymyxin variants. The U.S. National Institute of Allergy and Infectious Diseases (NIAID) has provided financial support to Prof. Roger Nation and Prof. Jian Li at Monash University (Melbourne, Australia) for their research on polymyxins. Similarly, the NIAID has provided financial support to Spero Therapeutics (Cambridge, MA, USA) and Qpex Biopharma (San Diego, CA, USA) for preclinical studies on SPR206[120−123] and QPX9003,[124] respectively, while CARB-X has extended its backing to MicuRx Pharmaceuticals, Inc. (Hayward, CA, USA) for the advancement of MRX-8.[125,126] All these new-generation polymyxins show enhanced activity against colistin-resistant strains, better pharmacokinetics, and lower toxicity.

CONCLUDING REMARKS AND PERSPECTIVES

The intrinsic resistance of Gram-negative bacteria remains one of the major obstacles to the development of effective antibacterial therapies. Central to this resistance is the OM, which acts both as a physical barrier limiting compound accumulation and as a highly specialized structure essential for

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bacterial viability. As highlighted throughout this review, recent advances have repositioned the Gram-negative OM from a passive obstacle to an actionable and pharmacologically tractable target.

Among the most promising strategies, direct inhibition of OM biogenesis pathways—such as the BAM complex, the Lpt machinery, and the Lol lipoprotein transport system—has yielded compelling proof-of-concept molecules. In particular, the discovery and subsequent optimization of darobactins and macrocyclic peptides targeting Lpt components demonstrate that high-affinity and selective inhibition of OM processes can translate into potent antibacterial activity and in vivo efficacy. These successes underscore the value of targeting conserved and essential envelope machineries that are accessible from the bacterial surface and less susceptible to classical permeability and efflux constraints.

In parallel, growing insights into the physicochemical determinants governing compound accumulation in Gram-negative bacteria have reshaped antibacterial drug discovery paradigms. The emergence of accumulation rules such as eNTRY and PASsagE highlights how subtle modulation of charge, rigidity, and molecular topology can dramatically influence intracellular exposure. These principles provide a rational framework for the design of antibiotics and, importantly, for the development of membrane-active potentiators capable of restoring the activity of otherwise ineffective drugs.

Looking forward, the most promising therapeutic avenues are likely to emerge from integrative strategies that combine OM targeting with intracellular inhibition. Antibiotic potentiation, whether achieved through membrane permeabilization, disruption of envelope biogenesis, or rational molecular hybridization, represents a particularly attractive approach to extend the lifespan of existing antibiotics while reducing the selective pressure associated with monotherapy. However, significant challenges remain, including the control of toxicity, the management of resistance development, and the translation of species-specific uptake rules into broadly effective agents.

Overall, targeting the Gram-negative OM offers a powerful and increasingly validated strategy to overcome intrinsic resistance. Continued progress will depend on the close integration of structural biology, microbiology, and medicinal chemistry to transform mechanistic insights into clinically viable antibacterial therapies.

In addition to biological and mechanistic constraints, the translational success of OM-targeting agents is further influenced by manufacturing feasibility and clinical safety. Many of the most advanced candidates, including macrocyclic peptides and chimeric molecules targeting BAM or Lpt components, pose challenges related to large-scale production, formulation, and cost, which may limit rapid industrialization and broader access.[127,128] Moreover, clinical experience has highlighted safety liabilities associated with certain membrane-active modalities, as exemplified by the discontinuation of systemic murepavadin therapy due to nephrotoxicity in phase III trials.[61] These considerations underscore the importance of integrating manufacturability and safety assessments early in the discovery process, particularly for large or complex molecular architectures, to ensure that promising OM-targeting strategies can be translated into clinically viable antibacterial therapies.

Importantly, many of the strategies discussed in this review remain constrained by species-specific OM architectures, differences in LPS composition, and variable susceptibility to resistance mechanisms, limiting their generalizability across Gram-negative pathogens. These constraints underscore the need to align target selection and molecular modality with pathogen-specific envelope biology rather than pursuing universally applicable solutions. Accordingly, successful future antibacterial discovery efforts will likely depend on a combination of tailored OM targeting, rational potentiation strategies, and careful management of resistance emergence.

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M.D. and M.M. wrote and approved the final manuscript.

Notes

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