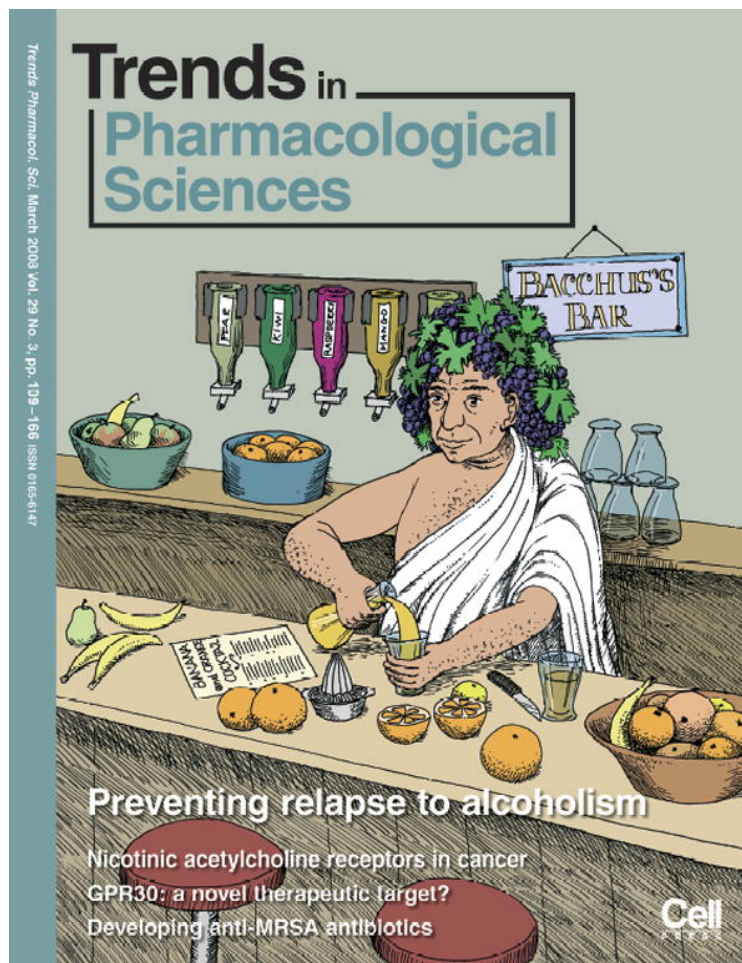


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# The bacterial envelope as a target for novel anti-MRSA antibiotics

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**Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-intermediate *S. aureus* (VISA) are spreading worldwide, making the search for antibiotics directed against new targets a high priority. Drugs that anchor in the bacterial membrane (e.g. ceragenins and lipopeptides) or that target the bacterial membrane and proteic (lipoglycopeptides) or lipidic (glycopepsipeptides) cell wall precursors seem to have the most potential because they show a fast and extensive bactericidal effect and are probably less prone to select for resistance owing to the difficulty in modifying their targets in a way that is compatible with bacterial survival. The efficacy of lipopeptides and lipoglycopeptides has been demonstrated in the treatment of skin and skin structure infections, and bacteremia caused by resistant *S. aureus*. Ceragenins and glycopepsipeptides are restricted to topical applications because of their unsatisfactory safety profile. The mode of action, pharmacological and microbiological properties and target indications of these anti-MRSA agents, which function by disturbing membrane integrity, are reviewed in this article.**

## Introduction

*Staphylococcus aureus* is a Gram-positive coccus that is enclosed in a thick cell wall (but no outer membrane, unlike Gram-negative bacteria); it was first described at the end of the 19th century in pus from human abscesses. Among *Staphylococcus* species, *S. aureus* is a major pathogen that is responsible for not only severe infections of the skin and skin structures but also life-threatening diseases such as pneumonia, endocarditis and bacteremia. *S. aureus* also causes protracted infections of bone (osteomyelitis) and joints (arthritis) and, because of its propensity to form biofilms on artificial materials, difficult-to-treat infections of catheters and other devices [1]. It is commonly seen inhabiting the nostrils and skin of ~30% of healthy people, from where it can cause major community- and healthcare-related infections. Invasive infections by this bacterium are associated with significant morbidity and mortality. *S. aureus* pathogenicity is multifactorial, and it resists and defeats many of the innate defense mechanisms (including immunoglobulins, defensins, phagocytosis, oxidative burst and iron-mediated mechanisms [1]). It also produces a large range

of tissue adhesins and secretes several virulence factors (enzymes and exotoxins such as hemolysins and Panton–Valentine leukocidin (PVL) [1]), which are responsible for the formation of abscesses, tissue necrosis, bacterial spreading, sepsis and shock [1]. Furthermore, *S. aureus* has acquired multiple resistance mechanisms to all conventional antibiotics (Box 1).

Originally highly susceptible to penicillin, clinical isolates of *S. aureus* were soon shown to produce penicillinase. The discovery of penicillinase-stable penicillins (such as methicillin) in the early 1960s provided temporary relief for clinicians, but methicillin-resistant *S. aureus* (MRSA) rapidly emerged thereafter by horizontal transfer of a mobile genetic element called the staphylococcal chromosome cassette *mec* (SCC*mec*), which carries the *mecA* operon. This gene encodes the modified penicillin-binding protein (PBP)2a, which maintains transpeptidase activity (ensuring its physiological function) while being poorly inhibited by  $\beta$ -lactams, making the bacterium resistant to this whole class of antibiotics. This mode of resistance has now spread to at least ten *S. aureus* lineages [2]. The prevalence of MRSA has increased on all continents, primarily in the hospital and long-term care settings [health-care-associated (HA)-MRSA strains] but also in the community [community-associated (CA)-MRSA strains], over the past decade [2]. Figure 1 shows the present situation by world regions based on international surveys [3,4]. In addition to being resistant to all conventional  $\beta$ -lactams, epidemic HA-MRSA strains tend to acquire other resistance determinants, resulting in co-resistance to fluoroquinolones, macrolides, lincosamides, streptogramins, aminoglycosides, rifampin and tetracyclines, whereas CA-MRSA remains, for the time being, susceptible to many non- $\beta$ -lactam antibiotics. However, it frequently harbors mobile genes encoding virulence-associated exotoxins that cause extended necroses (PVL being the most frequent) [5]. Risk factors for colonization by CA-MRSA include poor personal hygiene, crowded living conditions and close skin-to-skin contact [2].

Glycopeptides (e.g. vancomycin and teicoplanin) have long been the last-resort antibiotics against MRSA. These drugs inhibit peptidoglycan polymerization by binding to the D-Ala-D-Ala termini of precursors and impairing the subsequent reactions of transglycosylation and transpeptidation, thus compromising cell wall integrity [6] (Figure 2). Their large-scale use, mainly in the USA, led

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### Box 1. Conventional classes of antistaphylococcal antibiotics: pharmacological target, mode of action and main clinically meaningful mechanisms of resistance in *Staphylococcus aureus*

#### Inhibitors of cell wall synthesis

- $\beta$ -Lactams (penicillins and cephalosporins)

Target: penicillin-binding proteins (enzymes present at the bacterial surface that function as transpeptidases); inhibition of the reticulation of the pentapeptidic chains of peptidoglycan precursors  
Resistance: production of enzymes that inactivate the antibiotic ( $\beta$ -lactamases); production of a penicillin-binding protein (PBP2a) with reduced affinity for  $\beta$ -lactams (in MRSA) but that maintains its transpeptidase activity

- Glycopeptides

Target: D-Ala-D-Ala termini of peptidoglycan precursors; steric hindrance to prevent the activity of transpeptidases and transglycosidases involved in reticulation of peptidoglycan

Resistance: production of a thickened cell wall (in VISA); production of modified precursors ending in D-Ala-D-Lac, which have low affinity for glycopeptides (in VRSA)

- Bacitracin

Target: lipid carrier C55-isoprenyl pyrophosphate in the presence of  $Zn^{2+}$  (inner cell membrane lipid-carrier used to transport the peptidoglycan precursors of the cell wall from the inner to the outer leaflet of the membrane); inhibition of the recycling of this carrier  
Resistance: active efflux; increased synthesis of the target

#### Inhibitors of protein synthesis

- Macrolides, lincosamides, streptogramins

Target: 50S subunit of the ribosome; inhibition of elongation step in protein synthesis

Resistance: target modification (ribosomal methylation); active efflux of macrolides

- Tetracyclines

Target: 30S subunit of the ribosome; inhibition of the association of aminoacyl-trRNA to the acceptor site on the mRNA-ribosome complex

Resistance: active efflux of the antibiotic; ribosomal protection by production of proteins that prevent tetracyclines from accessing their target site

- Aminoglycosides

Target: 30S subunit of the ribosome; inhibition of the elongation step in protein synthesis and misreading of mRNA

Resistance: production of aminoglycoside-modifying enzymes

- Fusidic acid

Target: ribosome elongation factor-GTP plus inorganic phosphate complex; stabilization of this complex and blocking peptide elongation

Resistance: mutation in elongation factor

#### Inhibitors of nucleic acid synthesis

- Ansamycins

Target: RNA polymerase; inhibition of RNA synthesis

Resistance: target mutations

- Fluoroquinolones

Target: enzymes involved in DNA coiling (e.g. DNA gyrase and topoisomerase IV); stabilization of enzyme-DNA complex and generation of DNA breaks

Resistance: target mutation; active efflux of the drug

#### Metabolic inhibitors

- Sulfamides and diaminopyridines

Target: enzymes involved in the synthesis of folic acid (dihydropteroate synthase and dihydrofolate reductase); synergistic activity as inhibitors of folic acid synthesis

Resistance: target mutation

to the emergence of resistance. The most widespread organism (2.2% of MRSA strains [7]) is the so-called vancomycin-intermediate *S. aureus* (VISA), which has minimal inhibitory concentrations (MICs) of at least 4 mg/l. This strain displays a thickened cell wall, with an increased amount of free D-Ala-D-Ala termini, which can trap vancomycin and decrease its diffusion rate to its lipid II target at the septal tip of dividing bacteria [6,8]. Furthermore, this phenotype is often heterogeneous [heteroresistant *S. aureus* (hVISA)], requiring population analysis for proper detection. The co-resistance profile of (h)VISA isolates from the USA is similar to that of other HA-MRSA strains from which they derived [9] (Figure 1); in addition, VISA shows a decreased susceptibility to daptomycin because cell wall thickening prevents daptomycin from accessing its target at the membrane. Vancomycin treatment of severe (h)VISA infection is also associated with poor outcome [9]. Recently, isolates with high levels of resistance [vancomycin-resistant *S. aureus* (VRSA); vancomycin MIC >8 mg/l] have been described. These have acquired the VanA determinant of vancomycin-resistant *Enterococcus faecalis* [6].

The current prevalence of MRSA in most countries and the emergence of (h)VISA and VRSA create urgency in the development of new antibiotics that act against these strains. However, in the past 15 years, efforts – starting from existing classes – have led to only partial success (Box 2).

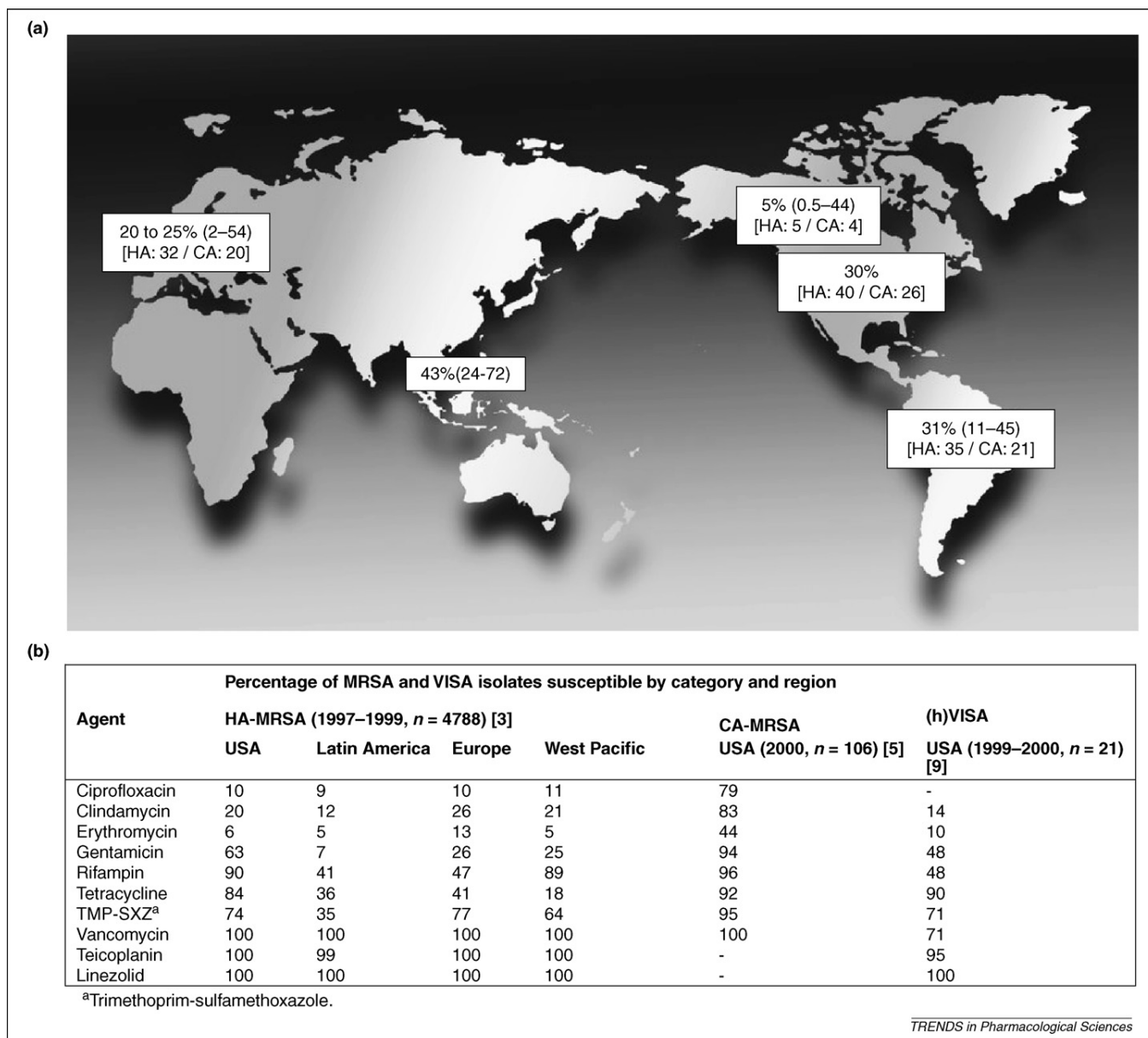
In this review, we focus on a series of amphipathic molecules with a general mode of action that has not yet been exploited clinically; most share the capacity to disturb membrane integrity. We examine them comparatively, starting with a description of their structures and modes of action, then analyze how these affect their microbiological and pharmacological properties and their target indications.

#### The bacterial envelope as a target for antibiotics

Gram-positive bacteria, including *S. aureus*, are limited by a single membrane that comprises mainly negatively charged phospholipids (phosphatidylglycerol and cardiolipin in *S. aureus* [10]) surrounded by a thick cell wall of peptidoglycan. Both structures are essential for cell survival, the membrane establishing a barrier of selective permeability and the cell wall protecting the bacteria from environmental factors. Whereas many antibiotics act on enzymes involved in peptidoglycan synthesis [11] (Figure 2), agents that alter membrane integrity are relatively novel in the clinical armamentarium, with the main difficulty involving finding molecules with selective toxicity for bacterial cells. This has been achieved using cationic agents or molecules that anchor in both the membrane and the cell wall.

Interest in the membrane as a new target has been highlighted by our understanding of the host innate immune defense mechanisms. For example, cationic antimicrobial peptides produced by the immune system (e.g. defensins) function primarily by permeabilizing the membrane of infective agents [12], although they might also inhibit polymerization steps in cell wall biosynthesis [13]. Antimicrobial peptides have several advantages [14] over

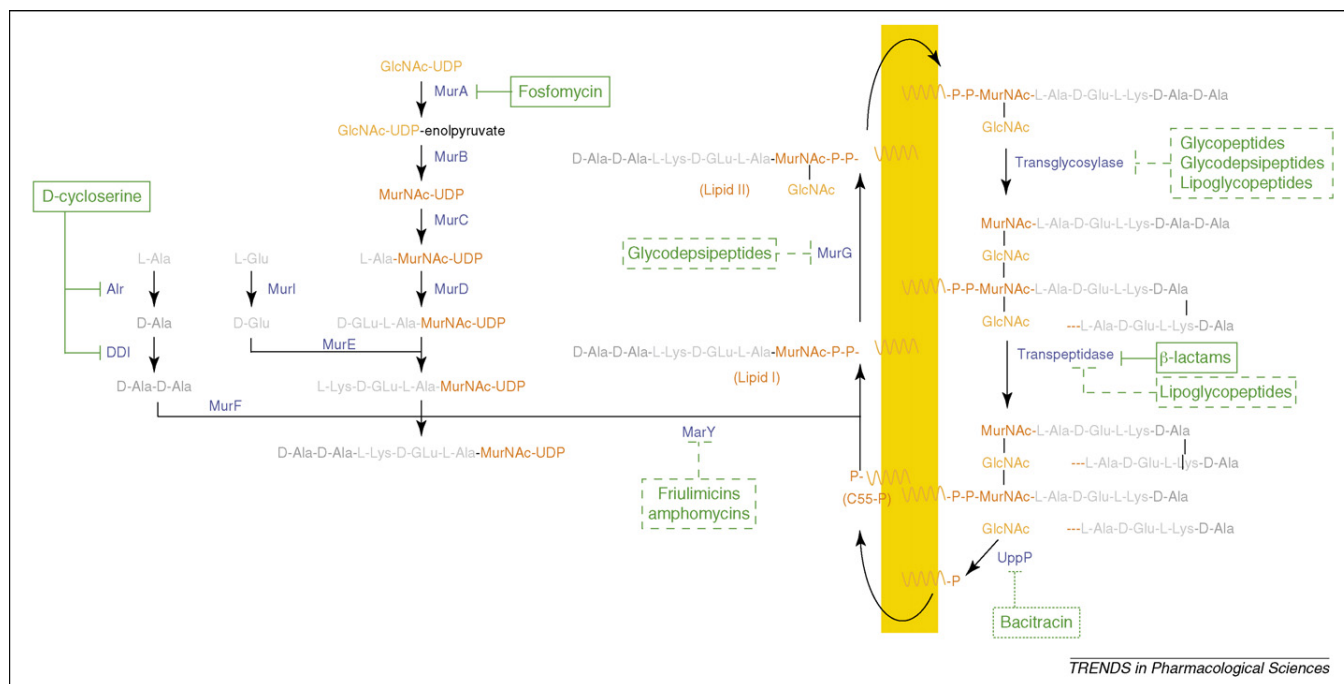




**Figure 1.** Worldwide epidemiology of MRSA and associated resistance mechanisms. (a) Prevalence by region of methicillin resistance among *Staphylococcus aureus* bloodstream isolates. Figures in round brackets correspond to the range observed for different countries in the same region; figures in square brackets correspond to the prevalence of HA-MRSA and CA-MRSA. Data are compiled from Ref. [3] (15 439 isolates collected worldwide between 1997 and 1999) and from Ref. [4] (50 759 isolates collected in Europe between 1999 and 2002). Although more recent global epidemiological data are not yet available, ongoing studies indicate an increasing trend for resistance, especially for CA-MRSA in the USA. (b) Antimicrobial susceptibility patterns of HA-MRSA and CA-MRSA strains, and strains with reduced susceptibility to vancomycin (hVISA or VISA).

conventional antibiotics: (i) a broad spectrum of activity; (ii) a highly bactericidal potency; (iii) no cross-resistance with existing drugs; and (iv) a low propensity for selecting for resistance. Selecting for resistance would indeed require a modification of the charge of membrane lipids or of cell wall constituents, which is poorly compatible with survival [15,16] (although transfer of L-lysine to phospholipids and of D-alanine to teichoic acids has been documented in *S. aureus* [16]). At present, a small number of peptides has been developed for clinical use (e.g. polymyxins) but therapeutic applications remain limited because of pharmacokinetic issues such as null oral bioavailability and short half-life [17]. However, the demonstration of their potent activity has stimulated the search for non-

peptidic, membrane-active antibiotics [14]. Of these, natural or semi-synthetic compounds such as ceragenins, lipopeptides, lipoglycopeptides and glycodepsipeptides represent the most promising advances in this direction. Each of these classes displays a slightly different mode of action, often implying a multiplicity of targets, making selection of resistance difficult and increasing specificity for bacterial cells. These molecules have a lipophilic tail and share common pharmacokinetic properties (e.g. the absence of oral absorption and prolonged retention in the organism). Figure 3 depicts the current view of the respective modes of action of these molecules and Figure 4 shows their chemical structures. Table 1 shows their main microbiological and pharmacological features.



**Figure 2.** Bacterial cell wall as a target for antibiotics. The pathway of peptidoglycan biosynthesis and current targets for antibiotics that inhibit cell wall synthesis are shown. Antibiotics that function by direct inhibition of enzymes are shown in unbroken boxes; those that inhibit enzymes by binding to their substrates are shown in broken boxes. Abbreviations: Alr, L-Ala racemase; D-Ala, D-alanine; DDI, D-Ala-D-Ala ligase; D-Glu, D-glutamate; L-Ala, L-alanine; L-Lys, L-lysine; MurNAc, N-acetylmuramic acid; UDP, uridine 5'-pyrophosphate; UDP-GlcNAc, UDP-N-acetylglucosamine; UppP, undecaprenyl pyrophosphatase.

**Box 2. New antistaphylococcal agents: pharmacological target, mode of action, circumvention of known resistance mechanisms**

**Inhibitors of cell wall synthesis**

- Anti-MRSA β-lactams [cephalosporins (ceftobiprole, ceftaroline)]  
Target: PBP2a  
Circumvented resistance: MRSA phenotype caused by the production of PBP2a

**Inhibitors of protein synthesis**

- Ketolides (telithromycin)  
Target: 50S subunit of the ribosome; same mode of action as macrolides  
Circumvented resistance: target modification (ribosomal methylation); active efflux (partial)
- Oxazolidinones\*  
Target: 50S subunit of the ribosome; inhibition of the formation of the initiation complex (assembly of the 30S and 50S subunit with mRNA and the first aminoacyl-tARN)  
Resistance: target modification
- Glycylcyclines (tigecycline)  
Target: 30S subunit of the ribosome (same mode of action as tetracyclines)  
Circumvented resistance: active efflux; ribosomal protection

**Metabolic inhibitors**

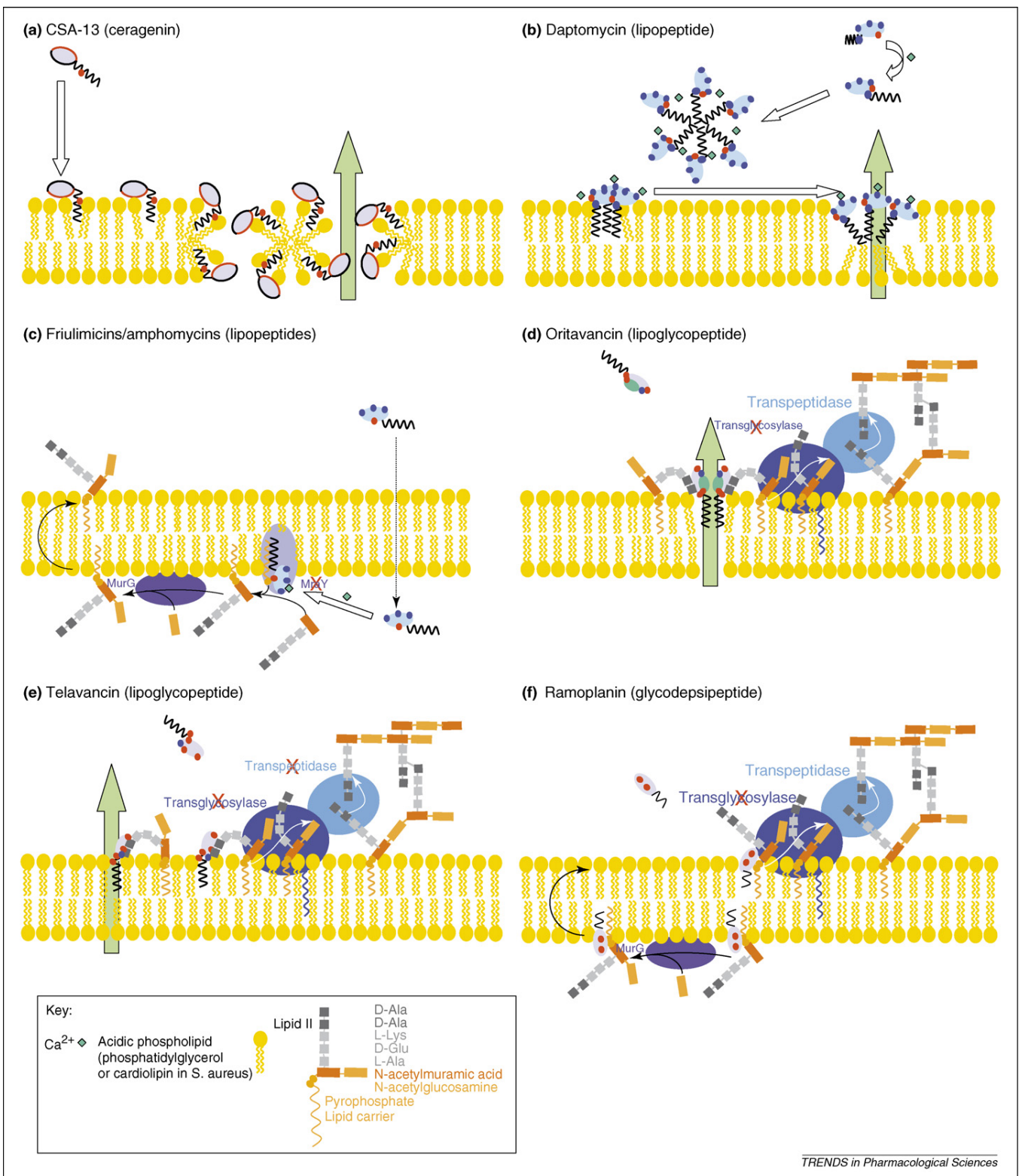
- New diaminopyridines (iclaprim)  
Target: dihydrofolate reductase (same mode of action as diaminopyridines)  
Circumvented resistance: target mutation

- FabI inhibitors\*  
Target: enoyl-acyl carrier protein reductase; inhibition of fatty acid synthesis  
Resistance: not yet described
- Peptide deformylase inhibitors\*  
Target: peptide deformylase; impairment of the hydrolysis of formylated N-terminal peptides necessary for protein maturation  
Resistance: not yet described

**Membrane-active agents**

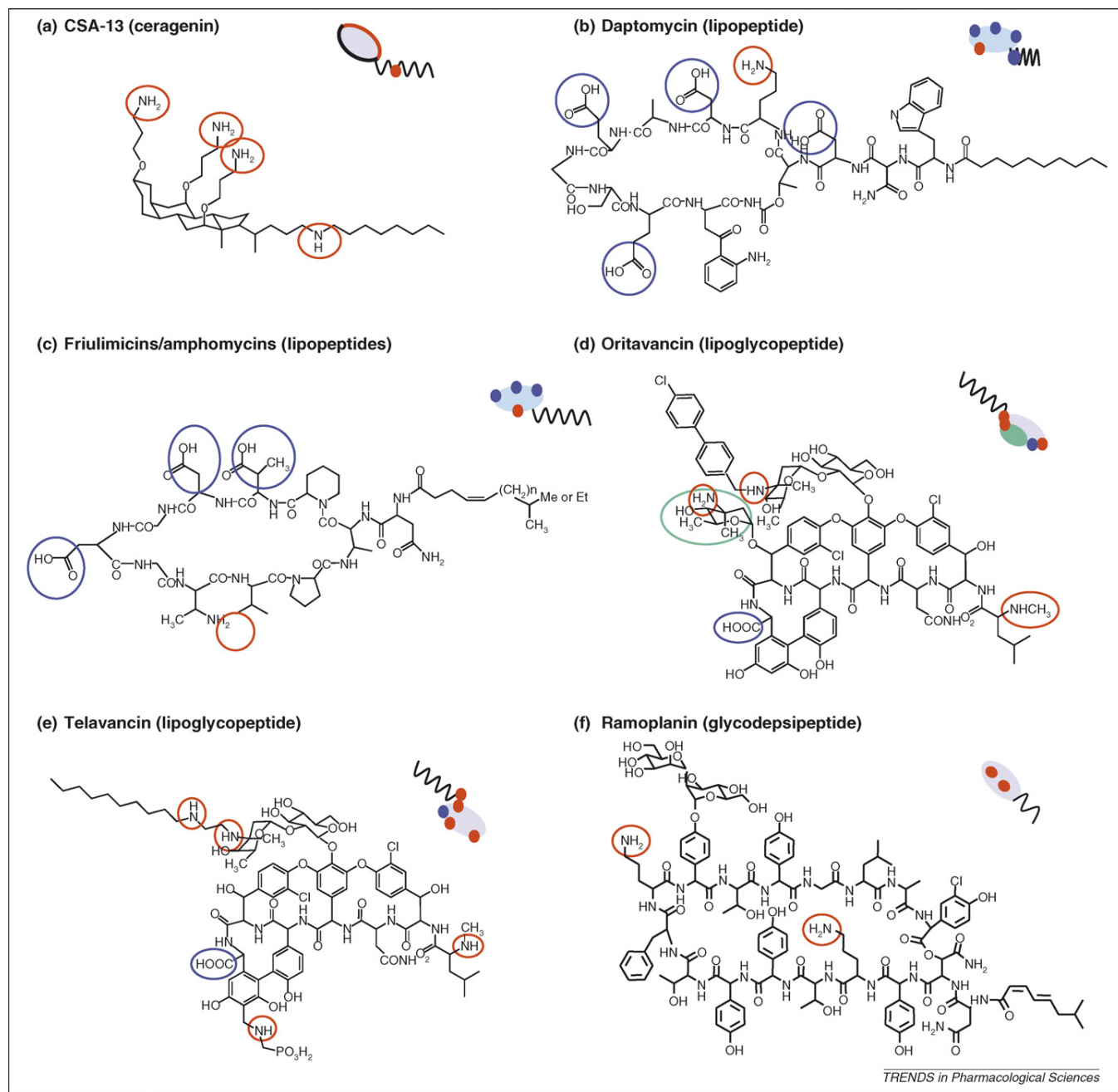
- Ceragenins\*  
Target: phosphatidylglycerol; membrane permeabilization and depolarization  
Resistance: not yet described
- Lipopeptides (daptomycin)\*  
Target: phosphatidylglycerol in the presence of Ca<sup>2+</sup>; membrane permeabilization and depolarization  
Resistance: mutation of a key enzyme involved in phosphatidylglycerol synthesis; reduced binding to bacterial membrane
- Friulimicins and amphomycins\*  
Target: lipid carrier C55-isoprenyl pyrophosphate; inhibition of MraY  
Resistance: not yet described
- Lipoglycopeptides\*  
Target: D-Ala-D-Ala termini of pentapeptidic precursors of peptidoglycan; membrane anchoring (lipid II?); membrane permeabilization and depolarization; inhibition of transglycosylase (and transpeptidase) activity  
Resistance: not yet described
- Glycodepsipeptides\*  
Target: lipid II; inhibition of transglycosylases and MurG  
Resistance: change in membrane charge

\* Because they are directed (in part for lipoglycopeptides) towards novel targets, these molecules are usually active against isolates that are resistant to the antibiotics mentioned in Box 1.



**Figure 3.** Current view of the mode of action of antibiotics that target the *Staphylococcus aureus* membrane. (See Figure 4 for the chemical structures of each antibiotic molecule). The schematic of each agent is shown to emphasize its amphipathic character, with basic functions circled in red and acidic functions in blue (the additional sugar in oritavancin is circled in green). Green arrows denote membrane permeabilization. The mode of interaction of each molecule with lipids or peptidoglycan precursors is shown based on current knowledge. **(a)** Ceragenins such as CSA-13 adopt a cationic, facially amphipathic structure in the presence of membrane and probably remove patches of lipids, causing membrane permeabilization and depolarization. **(b)** The lipopeptide daptomycin oligomerizes in the presence of Ca<sup>2+</sup>, changes conformation and forms micelles. When in contact with membranes, daptomycin inserts into the bilayer and undergoes a second conformational change, inducing positive membrane curvature and the leakage of K<sup>+</sup> and other cytosolic constituents. **(c)** Friulimicin and amphomycin lipopeptides bind to the undecaprenyl lipid carrier in the presence of Ca<sup>2+</sup>, which prevents the action of MraY (an enzyme that synthesizes lipid II). This mechanism assumes that these drugs can cross the membrane but their interactions with lipids have not yet been studied at the molecular level. **(d)** The lipoglycopeptide oritavancin has a high propensity for forming dimers (via its additional sugar moiety) and for anchoring in the membrane (via its lipophilic side chain). Owing to this cooperative binding, it not only binds tightly to the pentapeptidic (D-Ala-D-Ala) termini of peptidoglycan in vancomycin-susceptible strains, but remains also able to bind to the pentadepsipeptidic (D-Ala-D-Lac) termini in vancomycin-resistant strains,





**Figure 4.** Chemical structures of membrane-active antibiotics. The chemical structures of antibiotics that target the *Staphylococcus aureus* membrane: a ceragenin, CSA-13 (a); two lipopeptide subfamilies, daptomycin (b) and friulimicins (c) ( $n=5$  for friulimicin C;  $n=6$  for friulimicin A and B;  $n=7$  for friulimicin D) two lipoglycopeptides, oritavancin (d) and telavancin (e); and a glycodepsipeptide, ramoplanin A2 (f). The structure of each molecule is shown to emphasize its amphipathic character, with basic functions circled in red, and acid functions in blue (the additional sugar in oritavancin is circled in green). Schematic representations are the same as those used in Figure 3.

### Ceragenins

Ceragenins (Figure 4a) are cationic steroid antibiotics derived from cholic acids [18]. They adopt a cationic, facially amphipathic structure in the presence of lipids. They share similarities with cationic antimicrobial peptides, namely the capacity to cause membrane depolarization and permeabilization and to activate promoters of

genes responding to stress [15]. Therefore, it has been proposed that ceragenins could function as  $\alpha$ -helix-forming peptides, following a 'carpet' model (binding of the drug at the cell surface by electrostatic interactions, and removal of membrane patches when a sufficient concentration is reached) rather than a 'barrel-stave' model (aggregation into bundles and formation of semi-stable pores) [15]

thus preventing transglycosidases from accessing their substrates in both vancomycin-susceptible and vancomycin-resistant isolates. Bilayer disturbance also causes permeabilization of the membrane, which explains the more bactericidal effects of oritavancin compared with vancomycin. (e) The lipoglycopeptide telavancin also anchors in the membrane via its lipophilic side-chain, inducing membrane depolarization and permeabilization. Its binding to the pentapeptide or pentadepsipeptide of peptidoglycan precursors also inhibits transpeptidase and transglycosylase activity. As with oritavancin, telavancin is more bactericidal than vancomycin. (f) The glycodepsipeptide ramoplanin binds to the pyrophosphate group in lipid II and, with lower affinity, in lipid I, thereby impairing the activity of transglycosidases and, as a secondary target, of MurG.

Table 1. Pharmacological properties of new antibiotics that target *Staphylococcus aureus* envelope<sup>a</sup>

Drug	MIC (mg/l) <sup>b</sup>			Human dosage	Pharmacokinetic properties			Target MRSA indications	Refs
	MRSA <sup>c</sup>	VISA <sup>d</sup>	VRSA <sup>e</sup>		Protein binding (%)	Human C <sub>max</sub> (mg/l)	Human AUC (mg/h.l)		
Vancomycin	0.5–2 (1)	4–8	16, >128	15 mg/kg bid	10–55	50	260	Current recommendations: - severe infections by $\beta$ -lactam-resistant organisms - Gram-positive severe infections in patients allergic to $\beta$ -lactam - Prophylaxis in specific circumstances	[42,43,46]
CSA-13	(0.5)	(1)	1, 1	NA	NA	NA	NA	Topical applications for superficial infections	[20]
Daptomycin	0.12–1 (0.5)	0.25–2	0.125, 0.5	4–6 mg/kg/day	92	58–99	417–747	Registered for: - cSSSI - bacteremia - right-sided endocarditis	[43], [US package insert] <sup>f</sup>
Oritavancin	<0.004–1 (0.25) <sup>g</sup>	0.5–1 <sup>g</sup>	0.06, 0.5 <sup>g</sup>	3 mg/kg/day	90	31	152	- cSSSI (Phase III completed) - catheter-related bloodstream infection (Phase II completed)	[42]
Telavancin	0.06–1 (0.5)	0.125–1	2, 4	7.5–10 mg/kg/day	90 <sup>h</sup>	88	762 <sup>h</sup>	- cSSSI (registration in process) - nosocomial pneumonia (Phase III)	[42,43,46]
Dalbavancin	0.015–1 (0.25)	0.06–16	ND	1000 mg day 1 + 500 mg day 8	99	325	25 790	- cSSSI (Phase III completed)	[42,55]
Ramoplanin	(0.25)	ND	ND	ND	ND	ND	ND	- bacteremia Topical applications for decolonization, wound infection	[63,65]

<sup>a</sup>Vancomycin was used as a reference comparator. Abbreviations: AUC, area under the time–concentration curve; bid, *bis in die* (twice daily); cSSSI, complicated skin and skin structure infections [infections of the skin and underlying tissues (dermis, adipose tissue) such as post-surgical infections, traumatic wound infections, severe carbuncles for which surgical intervention is required (for debridement and drainage) or that is suspected to involve deeper soft tissues (fascia and/or muscle layers)]; NA, not applicable; ND, not determined.

<sup>b</sup>MIC ranges, with MIC<sub>90</sub> (MIC for 90% of the tested isolates) shown in brackets.

<sup>c</sup>Methicillin-resistant *S. aureus* (these bacteria are resistant to all  $\beta$ -lactams).

<sup>d</sup>Vancomycin-intermediate *S. aureus* [also known as glycopeptide-intermediate *S. aureus* (GISA) because these isolates also show reduced susceptibility to teicoplanin, a long-acting glycopeptide with other antibacterial properties similar to vancomycin, used in Europe]. These isolates are characterized by a thickened cell wall with increased peptidoglycan precursors displaying free D-Ala-D-Ala motifs, which bind to glycopeptides (resistance through target multiplication). Current susceptibility breakpoints [ $>2$  mg/l for the US Clinical and Laboratory Standard Institute (CLSI: <http://www.clsi.org>) and  $>4$  mg/l for the European Committee on Antibiotic Susceptibility Testing (EUCAST: <http://www.eucast.org>)] classify these organisms as intermediate because the use of vancomycin or teicoplanin to treat infections caused by them is of uncertain therapeutic effect.

<sup>e</sup>Vancomycin-resistant *S. aureus*. Resistance is related to a modification of the target of vancomycin by substitution of the terminal D-Ala of the peptidoglycan precursor with D-Lac. Individual values are shown for the first two strains, which were characterized and isolated in Michigan and Pennsylvania, USA.

<sup>f</sup>[www.cubicin.com/pdf/PrescribingInformation.pdf](http://www.cubicin.com/pdf/PrescribingInformation.pdf).

<sup>g</sup>MICs determined in the presence of 0.002% polysorbate (Tween 80), which prevents adhesion of the drug to plastics – see preliminary reports by Ahrin *et al.* [66] and Sahn *et al.* [67].

<sup>h</sup>Data for a dosage of 7.5 mg/kg/day; the dosage was increased to 10 mg/kg in Phase III trials upon protocol amendment and is used in present submission for FDA (<http://www.fda.gov/>) approval.

(Figure 3a). However, their action is probably more complex because there is no systematic correlation between the capacity of different ceragenins to permeabilize model membranes and their MICs [10]. The lipid composition of the bacterial membrane might have a crucial role in this context, with high potency observed towards Gram-positive bacteria (membrane rich in phosphatidylglycerol) and low potency towards Gram-negative bacteria (membrane rich in phosphatidylethanolamine) [10]. The specificity of ceragenins towards bacteria is suboptimal because they can also permeabilize eukaryotic cells [15,19], which severely limits their potential therapeutic applications.

Within this family, CSA-13 – which is in preclinical development by Ceragenix (see <http://www.ceragenix.com/> for further information about this molecule) – will probably be restricted to topical indications [15] or, after immobilizing at the surface of medical devices, for the prevention of biofilm formation [20].

### Lipopeptides

Lipopeptides are natural antibiotics that were discovered  $>50$  years ago [21]. Daptomycin (Figure 4b), the first registered member of this class and a fermentation product of *Streptomyces roseosporus* [22], consists of a cyclic, highly



hydrophilic depsipeptide attached to a decanoyl fatty acid chain (hence the name lipopeptide). Although globally polar, daptomycin undergoes a conformational change in the presence of  $\text{Ca}^{2+}$  that enhances its amphiphilicity by assembling the charged amino acids on one side of the molecule and exposing its lipophilic tail on the other side [23].  $\text{Ca}^{2+}$  also favors daptomycin oligomerization in micelle-like structures, with the lipid tails pointing inwards. In the presence of negatively charged membranes, daptomycin micelles go through a second structural transition that enables the interaction of the lipophilic tails with the membrane (Figure 3b). Daptomycin insertion in the outer leaflet of the bacterial membrane induces the leakage of cytosolic content, causing a rapid bactericidal effect. Daptomycin is active only on Gram-positive bacteria because it cannot cross the outer membrane of Gram-negative organisms. The specificity of daptomycin towards bacteria rather than eukaryotic cells is ensured by preferential interaction with phosphatidylglycerol [24]. Recent data, however, show that daptomycin is a substrate for P-glycoprotein, indicating that it also interacts with eukaryotic membranes but without affecting their integrity [25].

Resistance to daptomycin has already been described. Selected mutants show a gradual increase in MIC *in vitro*, associated with either reduced binding to the bacteria [26] or mutations that affect different genes – including *mprF*, which encodes the lysylphosphatidylglycerol synthetase involved in the synthesis of phosphatidylglycerol (also observed in daptomycin-resistant clinical isolates) [27]. VISA strains show a reduced susceptibility to daptomycin, probably because of impaired access of the drug to the membrane through the thickened cell wall [28].

The clinical development of daptomycin was stopped by its original discoverers (Eli Lilly: <http://www.lilly.com/>) in the early 1990s because of an unsuccessful Phase II trial and because of muscular toxicity [29]. Initiated by Cubist (<http://www.cubist.com/>) in 1997, pharmacodynamic studies have renewed clinical interest in the drug by demonstrating that muscular toxicity was observed upon prolonged exposure to the drug, whereas bactericidal activity was highly concentration dependent. Thus, it was clear that both safety and efficacy could be improved if the drug was administered once daily instead of twice daily, as was the case during the early development stage [29,30]. Once-daily daptomycin is now approved for the treatment of complicated infections of skin and skin structure [31] in North America, Europe and several other countries, and for *S. aureus* bacteremia and right-sided endocarditis [32,33] in the USA. A weakness of daptomycin, however, is its lack of efficacy in pneumonia because of inactivation by pulmonary surfactant (related to the  $\text{Ca}^{2+}$ -dependent formation of aggregates favored by the presence of a low proportion of phosphatidylglycerol in surfactant [34]).

Other lipopeptides such as friulimicins (Figure 4c) and amphomycins [21] share with daptomycin a  $\text{Ca}^{2+}$ -dependent mode of action, but they function by forming complexes with the lipid carrier C55-P in the presence of  $\text{Ca}^{2+}$ , thereby inhibiting MraY [35] (Figure 3c). Within this sub-family, MX-2401 is in preclinical development by Migenix (see <http://www.migenix.com/> for further information

about this molecule), with the advantage over daptomycin of not being inactivated by pulmonary surfactant.

### Lipoglycopeptides

In the early 1990s, Eli Lilly initiated a large-scale program of semi-synthesis – starting from vancomycin – that was aimed at discovering molecules unaffected by resistance mechanisms to conventional glycopeptides. This program showed that activity was reinforced if molecules formed dimers to improve target binding or could insert themselves in the membrane through a lipophilic side chain [6]. Three new lipoglycopeptides have now emerged; they show multiple modes of action and new pharmacological properties [6,36] and are in late stages of clinical development or await approval.

Oritavancin [discovered by Eli Lilly in 1996, then taken over successively by InterMune (<http://www.intermune.com/>) and Targanta (see <http://www.targanta.com/> for further information about this molecule)] differs from vancomycin by the addition of a 4-*epi*-vancosamine sugar, which increases dimer formation [37], and a chlorobiphenyl side-chain, which ensures membrane anchoring [38] (Figure 4d). Consequently, the drug remains capable of binding to a sufficient extent to the modified peptidoglycan precursors of vancomycin-resistant strains (in which the D-Ala-D-Ala motif is replaced with D-Ala-D-Lac or D-Ala-D-Ser [6]) and acquires a strong and fast bactericidal activity (correlating with membrane depolarization and permeabilization, in parallel with transglycosylase inhibition [38] [Figure 3d]). The structural changes also profoundly modify the pharmacokinetics of the drug, which shows a prolonged half-life and high retention in eukaryotic cells and tissues [6,39]. This makes oritavancin highly active against intracellular *S. aureus* [39], which is thought to have a major role in persistent or recurrent infections. In cell culture models, oritavancin induces a mixed lysosomal lipid-storage disorder because of its extensive accumulation [40] but *in vivo* data regarding whether this could be conducive to toxicity are lacking.

Telavancin (Figure 4e), which was discovered and developed at Theravance (see <http://www.theravance.com/> for further information about this molecule), differs from vancomycin by the presence of a lipophilic decylaminoethyl tail and a phosphonomethyl-aminomethyl substituent [41]. The latter compensates for the lipophilicity caused by the decylaminoethyl moiety, resulting in a shorter half-life and lower cellular accumulation compared with oritavancin [42,43]. Dimerization has not been shown for telavancin so far but, as with oritavancin, this antibiotic interacts with both native or modified peptidoglycan precursors and the membrane [a specific interaction with lipid II has recently been demonstrated (for review, see Ref. [44])], resulting in an inhibition of transglycosylation and transpeptidation reactions, and depolarization and permeabilization of bacterial membrane [45] (Figure 3e). This explains its intense bactericidal activity – which is maintained in VRSA, where binding to peptidoglycan precursors is probably weak [46]. As with oritavancin, telavancin is also highly active against intracellular *S. aureus*, regardless of the resistance phenotype [46].

Dalbavancin (see <http://www.pfizer.com/home/> for further information about this molecule) is a semi-synthetic derivative of a teicoplanin analog [6]. In contrast to the two previous lipoglycopeptides, it is not active against vancomycin-resistant strains and is also less bactericidal, indicating that its mode of action should not be too different from that of teicoplanin or vancomycin. It is, however, more potent, with MICs 2–32-times lower than those of vancomycin and teicoplanin. A unique property of dalbavancin is its unusually long half-life (Table 1), which enables a once-a-week administration [43]. Experimental research and clinical experience are needed to document fully several aspects of dalbavancin behavior related to its unique pharmacokinetic properties, such as the nature of the potential storage sites in the body, the risk of long-term toxicity issues and the emergence of resistance related to prolonged low serum levels.

Lipoglycopeptides act only on Gram-positive bacteria because they cannot cross the outer membrane of Gram-negative organisms. Their specificity of action towards prokaryotic cells is probably ensured by their dual interaction with peptidoglycan precursors and with membrane lipids. No resistance has been detected in clinical isolates so far, and attempts to select resistance *in vitro* are still unsuccessful, perhaps because of the multiple modes of action of these molecules.

The efficacy of all three lipoglycopeptides discussed has been demonstrated in pre-clinical and clinical studies [42]. The effectiveness of oritavancin in various animal models of staphylococcal (including endocarditis) and streptococcal (meningitis [47,48] and, recently, pneumonia) infections has been documented. It has now completed Phase III trials for MRSA-associated complicated skin and skin structure infections, in which it showed equivalent efficacy to vancomycin for shorter treatment duration [49]. Telavancin is efficacious in animal models of staphylococcal (including MRSA) soft-tissue infections, endocarditis, pneumonia, meningitis and bacteremia, including with VISA strains [50–52]. It has successfully completed Phase III trials for MRSA-associated complicated skin and skin structure infections [53,54] and its effects on hospital-acquired pneumonia caused by Gram-positive bacteria, with a focus on MRSA, are being studied [43]. Dalbavancin has completed Phase III studies for the treatment of MRSA-associated complicated skin and skin structure infections. An open-label, randomized, multicenter Phase II study also showed the efficacy of dalbavancin in catheter-related bloodstream infections [55].

Research is still active in the field of lipoglycopeptides, with new compounds under preclinical evaluation [36]. Interesting compounds that might emerge include heterodimers made of a glycopeptide covalently bound to a  $\beta$ -lactam [56].

### Glycodepsipeptides

Ramoplanin, the first glycodepsipeptide in clinical development (Figure 4f), is a fermentation product isolated from *Actinoplanes* sp. ATCC 33076 as a mixture of three constituents (A1, A2 and A3) [57]. [Discovered by Gruppo Lepetit Geranzano Research Laboratories, ramoplanin

has been licensed to Oscient Pharmaceuticals since 2001 (see <http://www.oscient.com/> for further information about this molecule.) Ramoplanin comprises a 49-membered macrocyclic depsipeptide substituted with a disaccharide and a lipid side-chain (variable among constituents; the A2 constituent has been selected for further development [58]). Ramoplanin functions by inhibiting MurG and transglycosylases. Its primary target, however, is probably lipid II, with binding occurring at the pyrophosphate group [44,59] (Figure 3f). The ring structure of the depsipeptide and the ornithine in position 10 of the macrocycle are crucial for antibacterial activity [60]. The lipid tail is also essential for activity, probably by directing ramoplanin to the membrane [58,61]. Owing to its low turnover rate, the availability of lipid II is a limiting step in peptidoglycan synthesis, making resistance hard to develop. Changes in membrane charge that confer resistance to other molecules targeting lipid II (e.g. lantibiotics, which are peptidic molecules produced by Gram-positive bacteria such as *Streptococcus* and *Streptomyces* to attack other Gram-positive bacteria; these molecules are not used in clinics) have, however, already been described [61].

Ramoplanin shows activity towards aerobic and anaerobic Gram-positive organisms, including staphylococci and enterococci that are resistant to conventional glycopeptides [62,63], but its clinical development is impaired by instability in the bloodstream and poor tolerance [58]. New derivatives in which the fatty acid chain is replaced with a 2-methylphenylacetic acid are being examined [64].

### Concluding remarks: what prospect for membrane-active antibiotics in the anti-MRSA arsenal?

Membrane-active antibiotics have several non-negligible advantages over other available anti-MRSA agents (e.g. vancomycin and linezolid). They are characterized by a strong, rapid and concentration-dependent bactericidal effect, which should assist the efficient eradication of infection over shorter treatment duration. They usually have low MICs for current MRSA isolates that display various resistance phenotypes (but the susceptibility of VISA must be critically assessed because of the poor performance of daptomycin in this context). Full resistance should be harder to select because of the multiplicity of targets and the difficulty bacteria could have in modifying their envelope in ways that avoid being a target and still remaining compatible with survival. The prolonged half-life of these membrane-active antibiotics also enables infrequent administration.

The main limitation of these agents rests with potential toxicity issues related either to imperfect specificity of action or to high levels of cellular accumulation. In addition, their physicochemical nature is incompatible with oral absorption, although this could be advantageous by potentially reducing inappropriate use when administration is confined to healthcare settings.

Future work in this field should, therefore, specifically address the following issues: (i) increased selectivity towards the bacterial membrane through an in-depth understanding of the mode of action of these drugs and their interaction(s) with their targets (to define true pharmaco-

phores); (ii) surveillance of resistance development and, if observed, determination of the underlying mechanisms (e.g. change in membrane composition, efflux and drug inactivation); and (iii) evaluation of efficacy in critical indications such as pneumonia, osteomyelitis and meningitis caused by multi-resistant organisms, in which conventional agents are now severely limited (improvement of drug distribution and expression of activity in the corresponding body compartments might be required).

In a broader context, it might also be essential to study whether these drugs can be successfully combined with other, more conventional antibiotics to enhance their activity and/or minimize the risk of the emergence of resistance. The concept of membrane-acting antibiotics should be further investigated to determine whether other molecules can be identified with activity on Gram-negative organisms (by targeting the outer or inner membranes), against which there is also an urgent need for novel molecules.

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#### References

- Moreillon, P. *et al.* (2005) *Staphylococcus aureus*. In *Principles and Practice of Infectious Diseases* (6th edn) (Mendell, G.E. *et al.*, eds), pp. 2321–2351, Elsevier
- Grundmann, H. *et al.* (2006) Emergence and resurgence of methicillin-resistant *Staphylococcus aureus* as a public-health threat. *Lancet* 368, 874–885
- Diekema, D.J. *et al.* (2001) Survey of infections due to *Staphylococcus* species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997–1999. *Clin. Infect. Dis.* 32 (Suppl. 2), S114–S132
- Tiemersma, E.W. *et al.* (2004) Methicillin-resistant *Staphylococcus aureus* in Europe, 1999–2002. *Emerg. Infect. Dis.* 10, 1627–1634
- Naimi, T.S. *et al.* (2003) Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. *JAMA* 290, 2976–2984
- Van Bambeke, F. *et al.* (2004) Glycopeptide antibiotics: from conventional molecules to new derivatives. *Drugs* 64, 913–936
- Liu, C. and Chambers, H.F. (2003) *Staphylococcus aureus* with heterogeneous resistance to vancomycin: epidemiology, clinical significance, and critical assessment of diagnostic methods. *Antimicrob. Agents Chemother.* 47, 3040–3045
- Pereira, P.M. *et al.* (2007) Fluorescence ratio imaging microscopy shows decreased access of vancomycin to cell wall synthetic sites in vancomycin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 51, 3627–3633
- Fridkin, S.K. *et al.* (2003) Epidemiological and microbiological characterization of infections caused by *Staphylococcus aureus* with reduced susceptibility to vancomycin, United States, 1997–2001. *Clin. Infect. Dis.* 36, 429–439
- Epand, R.F. *et al.* (2007) Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds (ceragenins). *Biochim. Biophys. Acta* 1768, 2500–2509
- Silver, L.L. (2006) Does the cell wall of bacteria remain a viable source of targets for novel antibiotics? *Biochem. Pharmacol.* 71, 996–1005
- Jenssen, H. *et al.* (2006) Peptide antimicrobial agents. *Clin. Microbiol. Rev.* 19, 491–511
- Sahl, H.G. *et al.* (2005) Mammalian defensins: structures and mechanism of antibiotic activity. *J. Leukoc. Biol.* 77, 466–475
- Peschel, A. and Sahl, H.G. (2006) The co-evolution of host cationic antimicrobial peptides and microbial resistance. *Nat. Rev. Microbiol.* 4, 529–536
- Savage, P.B. *et al.* (2002) Antibacterial properties of cationic steroid antibiotics. *FEMS Microbiol. Lett.* 217, 1–7
- Kraus, D. and Peschel, A. (2006) Molecular mechanisms of bacterial resistance to antimicrobial peptides. *Curr. Top. Microbiol. Immunol.* 306, 231–250
- Marr, A.K. *et al.* (2006) Antibacterial peptides for therapeutic use: obstacles and realistic outlook. *Curr. Opin. Pharmacol.* 6, 468–472
- Li, C. *et al.* (1999) Antimicrobial activities of amine- and guanidine-functionalized cholic acid derivatives. *Antimicrob. Agents Chemother.* 43, 1347–1349
- Ding, B. *et al.* (2002) Correlation of the antibacterial activities of cationic peptide antibiotics and cationic steroid antibiotics. *J. Med. Chem.* 45, 663–669
- Chin, J.N. *et al.* (2007) Antimicrobial activities of ceragenins against clinical isolates of resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 51, 1268–1273
- Baltz, R.H. *et al.* (2005) Natural products to drugs: daptomycin and related lipopeptide antibiotics. *Nat. Prod. Rep.* 22, 717–741
- Huber, F.M. *et al.* Eli Lilly and Co. A-21978C cyclic peptide derivatives. EP 178152 A2 19860416
- Jung, D. *et al.* (2004) Structural transitions as determinants of the action of the calcium-dependent antibiotic daptomycin. *Chem. Biol.* 11, 949–957
- Straus, S.K. and Hancock, R.E. (2006) Mode of action of the new antibiotic for Gram-positive pathogens daptomycin: comparison with cationic antimicrobial peptides and lipopeptides. *Biochim. Biophys. Acta* 1758, 1215–1223
- Lemaire, S. *et al.* (2007) Modulation of the cellular accumulation and intracellular activity of daptomycin towards phagocytized *Staphylococcus aureus* by the P-glycoprotein (MDR1) efflux transporter in human THP-1 macrophages and Madin–Darby canine kidney cells. *Antimicrob. Agents Chemother.* 51, 2748–2757
- Kaatz, G.W. *et al.* (2006) Mechanisms of daptomycin resistance in *Staphylococcus aureus*. *Int. J. Antimicrob. Agents* 28, 280–287
- Friedman, L. *et al.* (2006) Genetic changes that correlate with reduced susceptibility to daptomycin in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 50, 2137–2145
- Sakoulas, G. *et al.* (2006) Induction of daptomycin heterogeneous susceptibility in *Staphylococcus aureus* by exposure to vancomycin. *Antimicrob. Agents Chemother.* 50, 1581–1585
- Rybak, M.J. (2006) The efficacy and safety of daptomycin: first in a new class of antibiotics for Gram-positive bacteria. *Clin. Microbiol. Infect.* 12 (Suppl. 1), 24–32
- Dvorchik, B.H. *et al.* (2003) Daptomycin pharmacokinetics and safety following administration of escalating doses once daily to healthy subjects. *Antimicrob. Agents Chemother.* 47, 1318–1323
- Arbeit, R.D. *et al.* (2004) The safety and efficacy of daptomycin for the treatment of complicated skin and skin-structure infections. *Clin. Infect. Dis.* 38, 1673–1681
- Falagas, M.E. *et al.* (2007) Daptomycin for endocarditis and/or bacteraemia: a systematic review of the experimental and clinical evidence. *J. Antimicrob. Chemother.* 60, 7–19
- Fowler, V.G., Jr *et al.* (2006) Daptomycin versus standard therapy for bacteremia and endocarditis caused by *Staphylococcus aureus*. *N. Engl. J. Med.* 355, 653–665
- Silverman, J.A. *et al.* (2005) Inhibition of daptomycin by pulmonary surfactant: *in vitro* modeling and clinical impact. *J. Infect. Dis.* 191, 2149–2152
- Silver, L.L. (2003) Novel inhibitors of bacterial cell wall synthesis. *Curr. Opin. Microbiol.* 6, 431–438
- Kahne, D. *et al.* (2005) Glycopeptide and lipoglycopeptide antibiotics. *Chem. Rev.* 105, 425–448
- Cooper, R.D. *et al.* (1996) Reductive alkylation of glycopeptide antibiotics: synthesis and antibacterial activity. *J. Antibiot. (Tokyo)* 49, 575–581
- Allen, N.E. and Nicas, T.I. (2003) Mechanism of action of oritavancin and related glycopeptide antibiotics. *FEMS Microbiol. Rev.* 26, 511–532
- Van Bambeke, F. *et al.* (2004) Cellular pharmacokinetics and pharmacodynamics of the glycopeptide antibiotic oritavancin (LY333328) in a model of J774 mouse macrophages. *Antimicrob. Agents Chemother.* 48, 2853–2860
- Van Bambeke, F. *et al.* (2005) Mixed-lipid storage disorder induced in macrophages and fibroblasts by oritavancin (LY333328), a new



- glycopeptide antibiotic with exceptional cellular accumulation. *Antimicrob. Agents Chemother.* 49, 1695–1700
- 41 Judice, J.K. and Pace, J.L. (2003) Semi-synthetic glycopeptide antibacterials. *Bioorg. Med. Chem. Lett.* 13, 4165–4168
- 42 Van Bambeke, F. (2006) Glycopeptides and glycodepsipeptides in clinical development: a comparative review of their antibacterial spectrum, pharmacokinetics and clinical efficacy. *Curr. Opin. Investig. Drugs* 7, 740–749
- 43 Laohavaleeson, S. *et al.* (2007) Telavancin: a novel lipoglycopeptide for serious Gram-positive infections. *Expert Opin. Investig. Drugs* 16, 347–357
- 44 van Heijenoort, J. (2007) Lipid intermediates in the biosynthesis of bacterial peptidoglycan. *Microbiol. Mol. Biol. Rev.* 71, 620–635
- 45 Higgins, D.L. *et al.* (2005) Telavancin, a multifunctional lipoglycopeptide, disrupts both cell wall synthesis and cell membrane integrity in methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 49, 1127–1134
- 46 Barcia-Macay, M. *et al.* (2006) Evaluation of the extracellular and intracellular activities (human THP-1 macrophages) of telavancin versus vancomycin against methicillin-susceptible, methicillin-resistant, vancomycin-intermediate and vancomycin-resistant *Staphylococcus aureus*. *J. Antimicrob. Chemother.* 58, 1177–1184
- 47 Lefort, A. *et al.* (2000) Activity of LY333328 combined with gentamicin *in vitro* and in rabbit experimental endocarditis due to vancomycin-susceptible or -resistant *Enterococcus faecalis*. *Antimicrob. Agents Chemother.* 44, 3017–3021
- 48 Cabellos, C. *et al.* (2003) Experimental study of LY333328 (oritavancin), alone and in combination, in therapy of cephalosporin-resistant pneumococcal meningitis. *Antimicrob. Agents Chemother.* 47, 1907–1911
- 49 Ward, K.E. *et al.* (2006) Oritavancin – an investigational glycopeptide antibiotic. *Expert Opin. Investig. Drugs* 15, 417–429
- 50 Reyes, N. *et al.* (2006) Efficacy of telavancin in a murine model of bacteraemia induced by methicillin-resistant *Staphylococcus aureus*. *J. Antimicrob. Chemother.* 58, 462–465
- 51 Madrigal, A.G. *et al.* (2005) Efficacy of telavancin in a rabbit model of aortic valve endocarditis due to methicillin-resistant *Staphylococcus aureus* or vancomycin-intermediate *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 49, 3163–3165
- 52 Miro, J.M. *et al.* (2007) Efficacy of telavancin in the treatment of experimental endocarditis due to glycopeptide-intermediate *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 51, 2373–2377
- 53 Stryjewski, M.E. *et al.* (2006) Telavancin versus standard therapy for treatment of complicated skin and skin structure infections caused by Gram-positive bacteria: FAST 2 study. *Antimicrob. Agents Chemother.* 50, 862–867
- 54 Stryjewski, M.E. *et al.* (2005) Telavancin versus standard therapy for treatment of complicated skin and soft-tissue infections due to Gram-positive bacteria. *Clin. Infect. Dis.* 40, 1601–1607
- 55 Chen, A.Y. *et al.* (2007) Dalbavancin: a novel antimicrobial. *Int. J. Clin. Pract.* 61, 853–863
- 56 Pace, J.L. and Yang, G. (2006) Glycopeptides: update on an old successful antibiotic class. *Biochem. Pharmacol.* 71, 968–980
- 57 Cavalleri, B. *et al.* (1984) A-16686, a new antibiotic from *Actinoplanes*. I. Fermentation, isolation and preliminary physico-chemical characteristics. *J. Antibiot. (Tokyo)* 37, 309–317
- 58 Walker, S. *et al.* (2005) Chemistry and biology of ramoplanin: a lipoglycodepsipeptide with potent antibiotic activity. *Chem. Rev.* 105, 449–476
- 59 Cudic, P. *et al.* (2002) Complexation of peptidoglycan intermediates by the lipoglycodepsipeptide antibiotic ramoplanin: minimal structural requirements for intermolecular complexation and fibril formation. *Proc. Natl. Acad. Sci. U. S. A.* 99, 7384–7389
- 60 Nam, J. *et al.* (2007) Alanine scan of [L-dap(2)]ramoplanin A2 aglycon: assessment of the importance of each residue. *J. Am. Chem. Soc.* 129, 8747–8755
- 61 Breukink, E. and de Kruijff, B. (2006) Lipid II as a target for antibiotics. *Nat. Rev. Drug Discov.* 5, 321–332
- 62 Bozdogan, B. *et al.* (2003) Antibacterial susceptibility of a vancomycin-resistant *Staphylococcus aureus* strain isolated at the Hershey Medical Center. *J. Antimicrob. Chemother.* 52, 864–868
- 63 Farver, D.K. *et al.* (2005) Ramoplanin: a lipoglycodepsipeptide antibiotic. *Ann. Pharmacother.* 39, 863–868
- 64 Ciabatti, R. *et al.* (2007) Synthesis and preliminary biological characterization of new semisynthetic derivatives of ramoplanin. *J. Med. Chem.* 50, 3077–3085
- 65 McCafferty, D.G. *et al.* (2002) Chemistry and biology of the ramoplanin family of peptide antibiotics. *Biopolymers* 66, 261–284
- 66 Ahrin, F.F. *et al.* (2007) Effect of polysorbate-80 on oritavancin binding to plastic surfaces – implications for susceptibility testing. In *Programme and Abstracts of the 17th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) & 27th International Congress of Chemotherapy (ICC)*, Munich, Germany, abstract P-1112
- 67 Sahm, D.F. *et al.* (2007) *In vitro* activity profile of oritavancin against resistant staphylococcal populations from a recent surveillance initiative. In *Programme and Abstracts of the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Chicago, IL, USA, abstract E-1617