Multitarget Approaches against Multiresistant Superbugs

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Review

Multitarget Approaches against Multiresistant Superbugs

Declan Alan Gray and Michaela Wenzel*

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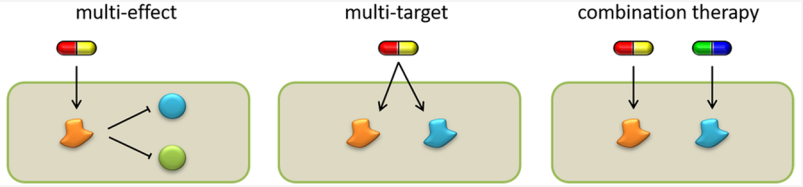

ABSTRACT: Despite efforts to develop new antibiotics, antibacterial resistance still develops too fast for drug discovery to keep pace. Often, resistance against a new drug develops even before it reaches the market. This continued resistance crisis has demonstrated that resistance to antibiotics with single protein targets develops too rapidly to be sustainable. Most successful long-established antibiotics target more than one molecule or possess targets, which are encoded by multiple genes. This realization has motivated a change in antibiotic development toward drug candidates with multiple targets. Some mechanisms of action presuppose multiple targets or at least multiple effects, such as targeting the cytoplasmic membrane or the carrier molecule bactoprenol phosphate and are therefore particularly promising. Moreover, combination therapy approaches are being developed to break antibiotic resistance or to sensitize bacteria to antibiotic action. In this Review, we provide an overview of antibacterial multitarget approaches and the mechanisms behind them.

KEYWORDS: *polypharmacology, multifunctional antibiotic, multiresistant bacteria, antibiotic combination therapy, synergy*

Antimicrobial resistance has developed into a global healthcare crisis that has culminated in the emergence of multidrug-resistant bacteria that are no longer treatable with any common antibiotic.[1] For example, recently emerging *Neisseria gonorrhoeae* strains resistant to third-generation carbapenems led to a number of untreatable gonorrhea infections in the UK.[2] Despite considerable investments, the development of innovative, resistance-breaking antibiotics still progresses too slowly.[3,4] For a long time, antibiotics with one specific protein target were highly sought after in drug design and screening efforts. Single target drugs were propagated as the ideal antibiotics with the argument that a high target specificity equals less side effects. This resulted in a predominantly genomic-driven approach to antibiotic discovery, where single protein targets were evaluated, while compounds with multiple or complex targets were regarded to be unspecific and unsuitable for further development. However, the last decades have shown that resistance to antibiotics with specific single protein targets develops too fast to be sustainable, and the realization emerged that candidates with multiple targets are worth pursuing for their slower resistance development rates.[5−7] In fact, long-established antibiotic drugs with great clinical success rarely target only one specific molecule. For example, β-lactam antibiotics typically target more than one transpeptidase, and quinolones inhibit both topoisomerases II and IV.[8,9] Antibiotics that truly have one single protein target, such as sulfonamides targeting an enzyme involved in folate synthesis or rifampicin targeting RNA polymerase, are famous for high resistance development and are therefore usually administered in combination regimes.[10,11]

Naturally occurring antibiotics often do not have strict single targets either. The most common class of natural antimicrobial substances are antimicrobial peptides, which occur in virtually all organisms. These molecules predominantly target complex bacterial structures, e.g., the cell membrane, the cell wall, or both.[12,13] Moreover, antibiotic producers often produce a mix of structurally related compounds. This is, for example, the case for gramicidin (contain gramicidin A−C), tyrothricin (contains gramicidin A−C and tyrocidine A−D), and polymyxins (contains multiple peptides with small structural variations depending on the polymyxin type).[14,15] Such small structural variations can result in slightly different target interactions[16−20] and may complicate resistance development by target alterations and antibiotic-cleaving or scavenging molecules. Naturally occurring mixes have been used in clinical settings with great success and in some cases are combined

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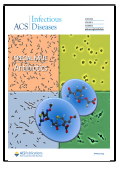

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Table 1. Examples for Clinically Used Antibiotics with Multiple Mechanisms of Action

antibiotic	targets	mechanism of action	ref
		Intrinsically Multi-effective	
daptomycin	phosphatidylglycerol, fluid lipid domains	binds to phosphatidylglycerol, inserts into fluid lipid domains that harbor the cell wall synthesis machinery, immediately inhibits cell wall and membrane synthesis; prolonged treatment results in partial membrane depolarization and impairs several other membrane-bound processes	25, 26
gramicidin S	membrane	induces membrane phase separation causing inhibition of cell envelope synthesis and cell division	20
vancomycin	lipid II	binds to lipid II, thereby inhibits peptidoglycan synthesis and depletes the pool of bactoprenol phosphate, additionally resulting in the inhibition of wall teichoic acid synthesis	27, 28
bacitracin	bactoprenol pyrophosphate	depletes the pool of bactoprenol phosphate resulting in inhibition of peptidoglycan and wall teichoic acid synthesis	29
nitrofurantoin	cellular macromolecules	generates reactive oxygen species, which damage cellular macromolecules including DNA and membrane lipids	30, 31
acyldepsipeptides	Clp protease	deregulates the Clp protease resulting in unspecific degradation of a variety of proteins	32, 33
bedaquiline	ATP synthase	inhibits ATP synthase, depleting the ATP pool and resulting in the inhibition of all energy-consuming cellular processes	34
		Multitarget	
penicillin	penicillin-binding proteins	inhibits multiple penicillin-binding proteins	8
ciprofloxacin	topoisomerase II and IV	inhibits topoisomerase II and IV	9
tetracycline	ribosome and membrane	blocks attachment of loaded aminoacyl tRNA to the A-site of the ribosome; also impairs membrane function	31, 35
polymyxin B	outer and inner membrane	permeabilizes both the outer and inner membrane of Gram-negative bacteria	36
tyrocidine	membrane and probably DNA	forms defined ion-conducting membrane pores; probably additionally binds to DNA	20, 37, 38
		Multifunctional	
clindamycin	50S rRNA	anti-inflammatory	39, 40
clofazimine	guanine	anti-inflammatory	41−43
dapsone	dihydropteroate synthase	anti-inflammatory and immunomodulatory	44, 45
macrolides	50S rRNA	anti-inflammatory and immunomodulatory	46−48
metronidazole	DNA	anti-inflammatory	49, 50
rifampicin	DNA-dependent RNA polymerase	anti-inflammatory and immunomodulatory	51, 52
tetracycline	ribosome and membrane	anti-inflammatory	53−55

with each other or other antibiotics like neomycin.[21,22] It is quite common in clinical practice to use antibiotic combinations for different reasons. For example, trimethoprim is combined with sulfonamides to prevent quick resistance development by a single target mutation, and erythromycin is often given together with penicillin for their synergistic effect.[23] Since more and more pathogens become resistant to individual antibiotics, it has become pivotal to thoroughly explore multitarget approaches to combat resistant bacteria. In this Review, we provide an overview of multitarget antibiotics and combination approaches that are in current clinical use or have a chance to be applied in clinical settings in the future.

PART 1: MULTITARGET COMPOUNDS

Generally, the ability of a single drug to interact with more than one specific target is called polypharmacology.[24] When talking about multitarget antibiotics, we distinguish between different levels of multitargeting. In this Review, we use the terminology intrinsically multi-effective, multitarget, and multifunctional. Intrinsically multi-effective compounds may have a single target, but this molecule or structure is involved in multiple processes, which are all affected by its inhibition. Multitarget antibiotics can be divided into multitarget compounds that target more than one isoenzyme or closely related proteins of the same pathway and multitarget compounds that bind to different molecules involved in separate cellular processes. Multifunctional compounds have a direct antibiotic target but an additional indirect antibacterial activity, e.g., immunomodulatory properties. Table 1 gives an overview of prominent antibiotics of these categories that are used in the clinic.

Intrinsically Multi-effective. The most common antimicrobial target, whose inhibition results in a multitude of cellular effects, is the cytoplasmic membrane. It is the target of many natural antibiotics, most prominently host defense peptides.[12] The cytoplasmic membrane harbors essential cellular processes, such as the respiratory chain and cell wall synthesis (Figure 1A), and many more important components, including nutrient uptake, secretion, and stress response systems. Thus, membrane-targeting antibiotics have the potential to (i) inhibit processes essential for survival, (ii) reduce bacterial fitness, (iii) impair virulence, and (iv) interfere with stress adaptation. Depending on how an antibiotic interferes with the membrane, few or a whole array of membrane-bound processes can be affected.[20] Several membrane-active antibiotics are used in the clinic, although only a handful are applied systemically. For some of them, their multiple effects on bacterial cells have been studied.

Many membrane-active antibiotics dissipate the membrane potential. This depolarization has a number of downstream effects due to the depletion of ATP and the subsequent impairment of ATP-dependent processes. Moreover, it leads to the displacement of peripheral membrane proteins that bind to the membrane surface by electrostatic interactions, such as the

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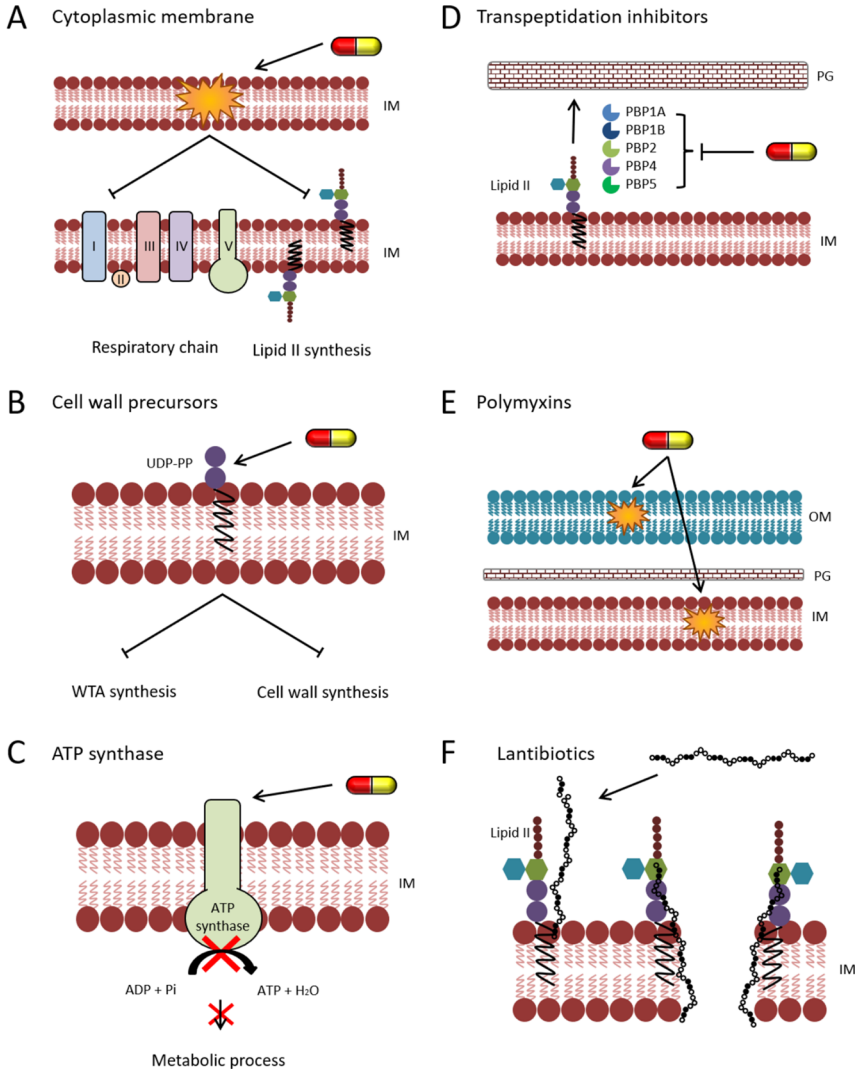

Figure 1. Examples for multiple mechanisms of action of antibiotics. (A–C) Antibiotics with intrinsically multi-effective properties. (A) Disrupting cytoplasmic membrane integrity, e.g., by gramicidin S, affects membrane-bound processes, most prominently respiration and lipid II synthesis. (B) Binding of antibiotics to lipid II or its carrier molecule undecaprenyl(pyro)phosphate (here: bacitracin binding UDP-PP) depletes the carrier pool, affecting both the synthesis of wall teichoic acids (WTA) and lipid II. (C) Inhibition of ATP synthase, e.g., by bedaquiline, leads to the depletion of the cellular ATP pool and thus inhibition of multiple metabolic processes. (D–F) Antibiotics with multiple targets. (D) β-Lactams (here imipenem) typically inhibit more than one penicillin-binding protein (PBP). (E) Polymyxins like polymyxin B or colistin disrupt both the outer and inner membrane of Gram-negative bacteria. (F) Type A lantibiotics like nisin bind to lipid II and use it as a docking molecule to form a transmembrane pore.

cell division proteins FtsA and MinD.[56,57] However, not all membrane effects can be explained by membrane depolarization. Thus, the membrane pore-forming peptides tyrocidine A and C not only dissipate the membrane potential and lead to leakage of ions and small molecules but also reduce membrane fluidity, diminish membrane domains, and affect the localization of several membrane-bound processes, including cell division, peptidoglycan synthesis, phospholipid synthesis, respiration, and ATP synthesis.[20] Such effects are not observed when the membrane potential is dissipated by specific ionophores.[56,57] The structurally similar peptide gramicidin S, which does not form distinct membrane pores and has milder effects on membrane potential and fluidity, only affects cell division and cell envelope synthesis proteins.[20,58] Daptomycin, which is one of the few systemically applied membrane-targeting antibiotics, has recently been described to interfere with both peptidoglycan and phospholipid synthesis by interfering with fluid membrane microdomains that harbor the lateral cell wall synthesis machinery.[25,31,59]

Similar results have been obtained for host defense peptides and experimental compounds that are not yet in clinical application. For example, human β-defensin 3 forms a dimeric raft-like structure with two α-helices anchoring it to the hydrocarbon layer and binds to negatively charged lipid head goups.[60] It has also been shown to bind the cell wall precursor lipid II with low affinity, probably due to electrostatic interactions with the pyrophosphate group.[61] Thus, the defensin is attracted to sites of active cell division and cell wall synthesis, where its presence disturbs the interactions of the complex peptidoglycan synthetic machinery, which was coined the "sand in the gearbox" effect.[61] The structurally similar human β-defensin 2 binds to distinct membrane foci at nascent cell division septa, where it disturbs the function of SecA and sortase A, which specifically localize to these sites

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and are involved in the secretion of virulence factors.[62] Other membrane-active compounds have been observed to have broader effects. For example, the membrane-disruptive peptides TC19 and TC84, which are derived from the microbicidal blood platelet protein thrombocidin,[63] lead to large-scale disruption of membrane organization and affect a multitude of cellular processes, including cell division, peptidoglycan synthesis, phospholipid synthesis, respiration, ATP synthesis, and spore outgrowth.[64−66] Such a broad panel of effects raises the following questions: which ones are due to the multifaceted mechanisms of the compounds and which are merely a consequence of cell death? It is therefore pivotal to examine the mechanism of a compound after short treatment times with sublethal concentrations.[4] The comparison of the effects observed under these conditions with those occurring at lethal concentrations or after prolonged treatment can give insight into which effects lead to cell death and which are a consequence thereof. In the case of the TC19 and TC84 peptides, membrane depolarization, rigidification, and phase separation were already observed at sublethal concentrations and increased at lethal concentrations, while other observations like protein delocalization and intracellular content leakage were mainly observed at lethal concentrations or after prolonged treatment.[64,65] This suggests that the primary mechanism of these peptides is the disruption of membrane organization. Similarly, depolarization and limited ion leakage are observed with daptomycin at high concentrations and long treatment times. However, at inhibitory, and even lethal, concentrations these effects do not occur, suggesting that increased membrane permeability is a consequence of cell death and not the primary mechanism by which it occurs.[67]

However, broad effects do not necessarily need to be a mere consequence of membrane disruption. Similar effects have been achieved by other compounds with quite creative molecular mechanisms. For example, the cyclic hexapeptide cWFW induces large-scale phase separation sorting peripheral and integral membrane proteins into two distinct domains,[68] and the plant-derived antibiotic candidate rhodomyrtone forms large membrane vesicles that irreversibly trap both peripheral and transmembrane proteins.[69]

Another molecular target that intrinsically presupposes multiple effects is the peptidoglycan precursor lipid II, which is the antibiotic target of the glycopeptide antibiotics vancomycin, telavancin, oritavancin, dalbavancin, and teicoplanin.[70−73] Lipid II is also the target of a variety of lantibiotics, most prominently the food preservative nisin. While nisin and other type A lantibiotics, such as gallidermin and epidermin, additionally target the cell membrane,[13,74,75] type B lantibiotics like mersacidin do not seem to have additional membrane effects.[74,76] Though the application of lantibiotics for antimicrobial coatings, e.g., for implants, is being explored,[77] none of them is currently used in a clinical setting. Lipid II is synthesized in the cytosol and attached to a lipid carrier molecule, undecaprenol phosphate, yielding the peptidoglycan building block lipid II. Lipid II flips over the membrane to the cell surface, and when the building block is incorporated into the cell wall, undecaprenyl phosphate flips back over the membrane to the cytosolic side, where it can undergo another cycle of peptidoglycan synthesis.[78] However, undecaprenyl phosphate is also used in the biosynthesis of wall teichoic acids, an important component of the Gram-positive bacterial cell wall.[78,79] The binding of antibiotics to lipid II not only directly prevents the incorporation of peptidoglycan building blocks into the cell wall but also depletes the undecaprenyl phosphate pool and thus concomitantly impairs the wall teichoic acid synthesis. Likewise, compounds that bind undecaprenyl phosphate (e.g., friulimicin B) or undecaprenol pyrophosphate (bacitracin) simultaneously inhibit both processes (Figure 1B). Moreover, cell wall synthesis is coupled to other cellular processes, most prominently cell division, and the binding of antibiotics to the sites of cell wall synthesis is likely to have additional "sand in the gearbox" effects.[61] Due to their typically large size and/or hydrophobic or amphipathic properties, membrane and cell wall-targeting antibiotics rarely cross the outer membrane of Gram-negative bacteria and are mostly used against and studied in Gram-positives. However, the general principle of the intrinsically multiple effects of such compounds is the same in all organisms.

These effects have also been predicted for other multiprotein machineries like the respiratory chain. Indeed, the inhibition of respiration and/or ATP synthase and subsequent depletion of ATP pools have been observed for several compounds, whose primary target is the cell membrane.[58,69,80−82] Membrane potential and respiration are closely intertwined, and the impairment of one can lead to the inhibition of the other.[83,84] Either way, both will have downstream effects on the performance of ATP synthase and the availability of ATP for important cellular processes (Figure 1C). Currently, no respiratory chain inhibitor is available as an antibiotic on the market, but with bedaquiline, the first ATP synthase inhibitor was marketed for the treatment of tuberculosis in 2012.[34] The impairment of the electron transport chain can also generate reactive oxygen species and thus cause oxidative stress.[85] This leads to widespread oxidative damage to cellular macromolecules, including DNA, proteins, and the cell membrane.[86] Oxidative damage is also a key mechanism of killing pathogenic bacteria by the immune system that is mediated by neutrophils.[87] While the generation of reactive oxygen species may be a side effect of membrane-targeting antibiotics, there is one antibiotic in the clinic that makes targeted use of oxidative stress. Nitrofurantoin, which is used against urinary tract infections, is a pro-drug that is activated by cellular nitrofuran reductases resulting in reactive intermediates that damage cellular macromolecules, especially DNA.[30,31]

Another mechanism that should be mentioned in this category is the deregulation of the ClpP protease by acyldepsipeptide antibiotics. ClpP takes part in both the general degradation of misfolded proteins and the regulated proteolysis of a range of substrates, including transcription factors and regulatory proteins. Acyldepsipeptides deactivate the tight control mechanisms that normally ensure that only such proteins that are either defective or supposed to be degraded as part of a regulatory cascade are degraded by ClpP. This leads to the uncontrolled proteolysis of intact cytosolic proteins and among them the key cell division protein FtsZ.[32,33,88]

Translation inhibitors that target the bacterial ribosome always bind to rRNAs, which are encoded in multiple copies in the bacterial genome.[5,89] While they only have one binding site, their resistance development rates are reduced, since simple point mutations are unlikely to affect all gene copies.[5] Translation inhibitors are also intrinsically multi-effective antibiotics, since an impaired ability to produce proteins hampers multiple cellular processes including the ability to elicit an appropriate stress response and thus prevents stress adaptation. Next to β-lactams, ribosome inhibitors constitute

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Table 2. Antibiotic Hybrid Molecules Currently under Clinical Development

compound	antibiotic 1 inhibited process 1	antibiotic 2 inhibited process 2	stage	ref
cadazolid	quinolone topoisomerases II and IV	oxazolidinone translation	phase III	122
cefilavancin (TD-1792)	vancomycin lipid II	cephalosporin PBPs	phase III	123
DNV3837 (MCB-3681)[a]	fluoroquinolone topoisomerases II and IV	oxazolidinone translation	phase II	124
TNP-2092 (CBR-2092)	rifamycin RNA polymerase	quinolone topoisomerases II and IV	phase II	120
TD-1607	glycopeptide lipid II	cephalosporin PBPs	phase I	125

[a]MCB-3681 was developed into the prodrug MBB-3837, which was renamed DNV3837 after Morphochem was acquired by Deinove.

the most successful antibiotic class in the clinic, underlining the potential of compounds with multiple targets.

Multiple Targets in the Same Pathway. Most of the antibiotics described above target one specific cellular structure, which itself is involved in different processes. However, there are also several antibiotics that bind to two or more distinct molecules. We will first discuss those compounds that target related proteins of the same pathway. The most extreme examples for this are the β-lactam antibiotics, which typically target more than one penicillin-binding protein (PBP) (Figure 1D).[8] For example, *Escherichia coli* has 8 PBPs, 6 of which are inhibited by penicillin G.[90−92] Other examples include quinolone antibiotics, which target both topoisomerase II and IV involved in DNA supercoiling and resolving DNA concatemers, respectively,[9] and the cell wall synthesis inhibitors fosfomycin and D-cycloserine. Gram-positive bacteria possess two copies of MurA, the first enzyme in the lipid II synthesis pathway, and fosfomycin inhibits both isoenzymes.[93] D-Cycloserine competitively inhibits both alanine racemase and D-ala-D-ala ligase, which converts L-alanine to the D-alanine dipeptide that is part of the peptidoglycan interpeptide bridge.[94] Similarly, platencin, a fatty acid synthesis inhibitor that attracted significant attention upon its discovery but then encountered several hurdles in preclinical development,[95] is a dual inhibitor of the FabF and FabH enzymes.[96,97]

Multiple Targets in Different Pathways. Several antibiotics have been found that inhibit two or more distinct targets that are not closely related components of the same pathway or process. Not only is it more difficult to acquire target-based resistance in two different molecules, but also bacteria need to react with two different stress responses to mitigate antibiotic action, hampering their capabilities for efficient stress adaptation; thus, these antibiotics have an additional advantage.

There are several examples for such compounds in the clinic, and new molecules with multiple targets are regularly discovered. One example are the polymyxins, in particular polymyxin B and colistin, which are used as last resort antibiotics against multiresistant Gram-negative infections.[98] These polypeptides target both the outer and the inner membrane of Gram-negative bacteria[99,100] (Figure 1E). Another example is clofazimine, which is used to treat leprosy.[101] This antibiotic binds to guanine bases in bacterial DNA but also increases phospholipase A1 activity, leading to toxic overproduction of lysophospholipids.[102] The atypical tetracycline chelocardin, which has recently attracted renewed interest for clinical development,[103,104] inhibits the bacterial ribosome and additionally depolarizes the cytoplasmic membrane.[105] Tetracycline itself, while not leading to depolarization, has recently been shown to disturb membrane organization in addition to translation inhibition.[31] The same was observed for anhydrotetracycline, suggesting that this dual activity could be a general activity of the tetracycline class.[31] It would be reasonable to assume that membrane activity may be a general consequence of translation inhibition, since ribosomes associate with the cell membrane to couple translation to protein secretion.[106] However, other ribosome inhibitors did not visibly disturb the cell membrane, and a ribosomal protein mutant that diminishes tetracycline binding to the ribosome showed the same tetracycline-induced membrane effects as the wild-type.[31] These findings suggest that the membrane activity of tetracycline is independent from translation inhibition and thus a separate secondary target. Similarly, tyrocidines, which primarily disrupt cytoplasmic membrane integrity, have long ago been proposed to bind DNA as secondary target. This notion, which was derived from test tube interactions of the peptides with isolated DNA,[38] was only recently supported by *in vivo* experiments.[20]

Another very prominent example are antibiotics with a dual mechanism on cell wall synthesis and the cell membrane. This is relatively common, since cell wall synthesis inhibitors often target membrane-bound steps of this pathway. Thus, both targets are in close proximity. The most prominent example for this is the lantibiotic nisin, which is not used clinically but is a common food preservative (Figure 1F). Nisin has a two-step mechanism of action. It first establishes contact with lipid II, inhibiting peptidoglycan synthesis. It then inserts into the lipid bilayer to form a transmembrane pore.[107−109] More recently, it was found that nisin also binds to undecaprenyl phosphate, adding another structure to its list of molecular targets.[110] While nisin is able to bind to negatively charged lipids in the absence of lipid II or undecaprenyl phosphate,[111] it needs the lipid-coupled cell wall precursor as a docking molecule to form a transmembrane pore. Other lantibiotics like gallidermin can impair both targets independently.[112] This property of lantibiotics also inspired the development of dual-function vancomycin derivatives. Telavancin and oritavancin carry a hydrocarbon tail, which allows them to insert into the cell membrane after binding lipid II. Thereby, they disrupt the permeability barrier, a feature that vancomycin is lacking and that restores activity against strains with high-level vancomycin resistance.[7,71] Daptomycin could be included in this group as well, since it likewise inserts into the cell membrane and impairs cell wall synthesis.[25] However, at least to our current knowledge, it only binds a single molecular target, namely, phosphatidylglycerol-containing lipids.[113−116]

Designer Dual-Target Compounds. Inspired by the clinical success of these multitarget antibiotics, approaches have been undertaken to develop dual-target molecules. These can either be stable hybrids combining two functions in one molecule or cleavable compounds, whereby the prodrug has a different target than the cleavage product.[117,118] In the past, cleavable compounds have essentially behaved like simple prodrugs for the cleavage product rather than eliciting their intended dual effects. Hence, newer efforts rather focus on

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stable hybrids.[118−120] These approaches have been recently reviewed in detail,[117,121] but we want to give a brief overview of the molecules that are currently underway in clinical development (Table 2).

Cadazolid is a stable hybrid of a quinolone and oxazolidinone and currently in phase III clinical trials for the treatment of *Clostridium difficile* infections.[122,126] It primarily inhibits translation but also topoisomerase activity and shows low resistance development rates.[127] Cefilavancin is a stable hybrid molecule of vancomycin and a third-generation cephalosporin, which is currently undergoing phase III clinical trials for complicated skin and soft tissue infections.[121] This hybrid compound is active against both methicillin and vancomycin-resistant *Staphylococcus aureus*, yet it has not been verified to which extent it inhibits lipid II and PBPs.[128,129] MCB-3681 is a stable fluoroquinolone−oxazolidinone hybrid similar to cadazolid.[124] It is active against strains that are resistant to both ciprofloxacin and linezolid.[130] MCB-3681 was scheduled to undergo phase II clinical trials since 2015 and was granted fast track status by the FDA in 2016.[124] Instead, it was further developed into a prodrug molecule, MCB-3837. In 2018, its developing company Morphochem was acquired by Deinove, and the compound, now under the name DNV3837, finally went into phase II clinical trials for treatment of *C. difficile* infections in 2019.[131] The study is planned to be completed in the summer of 2020.[132] TNP-2092 is a stable rifamycin−quinolone hybrid currently in phase II trials for prosthetic joint infections.[120] It is active against a range of quinolone and rifamycin-resistant strains.[120] TNP-2092 is also being evaluated against *Helicobacter pylori* infections yet at a preclinical stage.[133] TD-1607 is a stable glycopeptide−cephalosporin hybrid similar to cefilavancin. Two phase I clinical trials have been completed, yet results remain to be published.[121,125]

A different hybrid approach was followed by the company Visterra, who developed an ultranarrow spectrum hybrid molecule against *P. aeruginosa* infections by coupling a specific antibody that targets cell surface glycan molecules with an antimicrobial peptide.[134] Here, the hybrid molecule does not possess two antibacterial targets, but the antibody strategy allows more targeted treatment by increasing the local peptide concentration at the cell surface and bringing the antibiotic group close to its target.

Another approach to new multifunctional antibiotic candidates against Gram-negative bacteria based on the polymyxin lead structure has recently been published.[135] These compounds are chimeric peptidomimetics combining features of polymyxin A with the outer membrane protein-targeting cyclic peptide murepavadin. They permeabilize the outer membrane by targeting lipopolysaccharides, inhibit the outer membrane protein complex Bam, which is involved in the folding and insertion of β-barrel proteins in the outer membrane, and permeabilize the inner membrane.[135] One of these compounds is currently undergoing preclinical toxicology studies.[135]

It will be exciting to see whether these hybrid molecules will receive FDA approval and how they will ultimately perform in the clinic.

Multifunctional Compounds. The last category of multitarget molecules that we want to discuss are multifunctional compounds, which in addition to their antibacterial properties possess a second, unrelated activity that is beneficial for treatment. Most prominently, such compounds may have anti-inflammatory or immunomodulatory properties (Table 1). This is well-described for a range of antibiotics, and some are even used to treat conditions unrelated to infections due to these properties. This is, for example, the case for macrolides, which are used to treat diffuse panbronchiolitis,[47] dapsone, which has anti-inflammatory and immunomodulatory effects and is, for example, used against dermatitis herpetiformis,[136] or rifampicin, used against pruritus caused by primary biliary cholangitis.[137] For other indications, the dual effects of these compounds are crucial for their therapeutic efficacy. For example, clindamycin is used to treat acne for both its direct antibacterial action and its ability to decrease swelling and inflammation by modulating cytokine production.[40,138] Acne is a very common indication for combining antibacterial and anti-inflammatory activities. Other antibiotics with this dual function are metronidazole, tetracycline, macrolides, and dapsone, which are all used to treat acne.[48] Clofazimine, which is used against leprosy, already has two distinct antibacterial targets, guanine bases and phospholipase A2.[102] In addition to these activities, it also reduces neutrophil mobility, which is beneficial for treating patients with a type 2 lepra reaction characterized by strong inflammation.[139,140] The range of antibiotics with immunomodulatory and anti-inflammatory effects and the detailed use of these compounds have been thoroughly reviewed before[48,141−143] and should not be described here in more detail. However, it should be noted that daptomycin has been implicated to have immunomodulatory effects as well.[144] Daptomycin already has multiple effects on bacterial cells, and both tetracycline and clofazimine have two distinct antibacterial targets. This illustrates that multiple activities are quite common in clinically successful antibiotics.

PART 2: ANTIBIOTIC COMBINATIONS

Combination regimes are nowadays quite common in the clinic, normally to potentiate activity against multiresistant strains and prevent resistance development. A common regime is, for example, the combination of sulfonamides with trimethoprim in urinary tract infections (Figure 2).

Table 3 gives an overview of well-characterized antibiotic combinations and their effects. A significant amount of research is being conducted to find combinations that are more effective than monotherapy or that are resistance-breaking. When two antibiotics are combined, they are usually either indifferent in their activity or more effective in killing

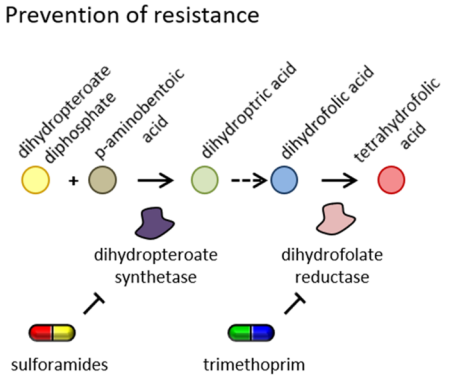

Figure 2. Prevention of resistance development by combination therapy. Sulfonamide antibiotics are typically given together with trimethoprim to prevent fast target mutation of a single enzyme.

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Table 3. Examples for Well-Characterized Antibiotic Combinations[a]

antibiotic 1	antibiotic 2	mechanism of combination	ref
		synergistic	
D-cycloserine inhibits peptidoglycan synthesis	epigallocatechin gallate binds to and disrupts the peptidoglycan layer	D-cycloserine inhibits lipid II synthesis, and epigallocatechin gallate disrupts cell wall peptidoglycan	149
ampicillin[b] inhibition of PBPs	daptomycin inhibition of membrane and cell wall synthesis	daptomycin affects membrane organization, which might interfere with the function of PBPs; it also inhibits lipid II synthesis by abolishing membrane binding of MurG	25, 150, 151
rifampicin[b] RNA synthase inhibition	fusidic acid ribosome inhibition	mechanism unknown, but it has been observed *in vitro* that DNA-dependent RNA-polymerase is inhibited by the elongation factor T	152−155
erythromycin[b] ribosome inhibition	penicillin inhibition of PBPs	inhibition of translation might deplete β-lactamases	153, 156, 157
		additive	
ampicillin[b] inhibition of PBPs	imipenem inhibition of PBPs	both antibiotics bind to the same site of PBP2A but with low affinity	150, 158, 159
azithromycin[b] ribosome inhibition	imipenem inhibition of PBPs	inhibition of translation might deplete PBPs, requiring lower doses of imipenem	160, 161
		indifferent	
sulphamethoxazole[b] inhibits dihydropteroate synthetase	trimethoprim inhibits dihydrofolate reductase	both compounds target folate synthesis but at different steps; a combination is given to prevent rapid resistance development to a single drug rather than increase activity	162−164
		potentiative	
amoxicilline[b] inhibition of PBPs	clavulanate β-lactamase inhibitor	clavulanate inhibits the β-lactamase that degrades amoxicilline	165, 166

[a]PBP: penicillin-binding protein. [b]Combination in clinical use.

bacteria due to additive effects. However, if the effects of the combination exceed the expected additive effects, the combination is called synergistic. This is, for example, the case for daptomycin and β-lactams.[145] The opposite effect is called antagonistic and is observed, e.g., with the antifungal drugs amphotericin B and ravuconazole.[146] Synergistic drug combinations are highly sought after, since they allow one to lower drug doses and thus may reduce side effects. A different approach to combination strategies is using potentiators, sometimes also called antibiotic adjuvants. These are compounds that by themselves have little or no antibacterial activity but enhance the effects of antibiotics when administered in combination. This is, for example, the case for resistance-breaking compounds like β-lactamase inhibitors.[147] Such compounds typically target either acquired or intrinsic antibiotic resistance mechanisms. There have also been approaches to develop inhibitors of horizontal gene transfer to prevent the spread of resistance genes.[148] In the following, we will discuss these different approaches to antibiotic combination therapy.

Antibiotic Synergy. Synergistic compound combinations are known against Gram-positive and Gram-negative bacteria and mycobacteria. However, the exact mechanisms of antibiotic synergy are mostly unknown. While there have been attempts to explain synergy, these have rarely been experimentally proven. However, there are four widely accepted categories of possible synergistic mechanisms for which examples are known (Figure 3).

One possible synergistic mechanism is the inhibition of the same target molecule at multiple binding sites (Figure 3A). An example for this is the synergy of plectasin with teicoplanin and dalbavancin, which all bind the peptidoglycan precursor lipid II.[167] However, this is not a universal phenomenon, and other antibiotics that target the same molecule might only have additive effects. This is, for example, the case for the PBP inhibitors ampicillin and imipenem.[150] Another possibility for synergy is that two compounds inhibit independent targets in the same pathway (Figure 3B). This is for example the case for D-cycloserine, an antituberculosis drug that inhibits an intracellular step of peptidoglycan synthesis,[168] and epigallocatechin gallate, a compound found in tea that is thought to disrupt cell wall peptidoglycan.[149,169] Epigallocatechin gallate also acts synergistically with β-lactam antibiotics[149] and inhibits β-lactamase activity.[170] Another example for this synergistic mechanism is again plectasin, which also acts synergistically with the glycosyltransferase inhibitor moenomycin.[167] However, plectasin does not show synergy with other cell wall synthesis inhibitors like vancomycin or penicillin G, demonstrating that the exact mechanisms underlying synergy are little understood.[167] Synergy can also be observed between compounds that inhibit related processes (Figure 3C). This can often be observed for antibiotics that target cell wall synthesis and membrane-active compounds, e.g., β-lactams and daptomycin[150,171,172] or the cationic antimicrobial peptide LL-37 and the lipid II-binding antibiotic teicoplanin.[173] In some cases, synergy is also observed between antibiotics with different targets in unrelated pathways as observed with penicillin and the ribosome inhibitor erythromycin. Here, it has been suggested that the depletion of penicillin-binding proteins by translation inhibition might be the underlying mechanism.[157] However, a similar combination of azithromycin (ribosome inhibitor) and imipenem (β-lactam) only results in additive effects.[174] The last documented mechanism underlying antibiotic synergy is the improvement of target accessibility (Figure 3D). This is, for example, observed with the outer membrane-permeabilizing peptide colistin in combination with drugs that have inner membrane-bound or cytosolic targets, such as the β-lactam meropenem, the ribosome inhibitor minocycline, and fosfomycin, which inhibits the first enzyme in the lipid II pathway.[175] This is an important anti-Gram-negative strategy that will be discussed in more detail in the following chapter. Antibiotic synergy has attracted significant attention over the last years, and efforts are being undertaken to discover new synergistic combinations that can be useful for clinical applications.[176−180]

Permeabilizers. In addition to synergistic antibiotic combinations, potentiative combination approaches have recently attracted increased attention. Potentiators typically target intrinsic or acquired antibiotic resistance mechanisms to sensitize or resensitize bacteria to existing antibiotics (Figure 4).

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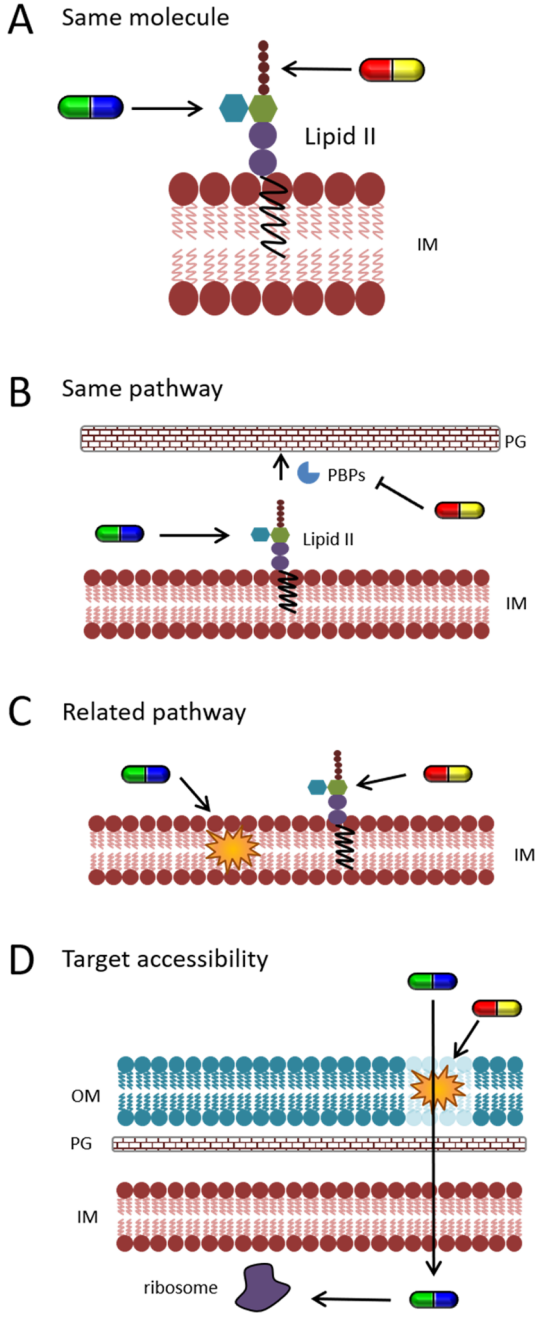

Figure 3. Mechanisms of synergy. (A) Targeting the same molecule (here, plectasin in red−yellow and dalbavancin in green−blue), (B) targeting the same pathway (here, plectasin in red−yellow and moenomycin in green−blue), (C) targeting a related process (here, LL-37 in green−blue and teicoplanin in red−yellow), and (D) improving target accessibility (here, colistin in red−yellow and minocycline in green−blue).

The advantage of these molecules is that they can potentially increase the activity of a whole array of existing antibiotics. One class of potentiators are cell envelope permeabilizers, especially outer membrane-permeabilizing compounds (Figure 4A). These are particularly interesting, since the outer membrane is the main reason why most antibiotics are ineffective against Gram-negative bacteria. The inactivation of this permeability barrier could make almost the whole array of current antibiotic drugs available for treating these infections, instead of the handful that we have at our disposal now. Two antibiotics in current clinical use are known to target the outer membrane of Gram-negative bacteria, colistin and polymyxin B. Both of them display synergy with antibiotics with intracellular targets, which can at least partially be attributed to increased outer membrane permeability.[175,181,182] Many other outer membrane-permeabilizing agents are known, for example, chelators like EDTA, metal ions, and certain antimicrobial peptides, but many of these have little clinical promise.[183−188] However, several compounds have recently been described that could make the step into clinical practice in the future. For example, the antiprotozoal drug pentamidine has been identified as an outer membrane-permeabilizing agent. It was shown to potentiate the activity of typical Gram-positive-only drugs against Gram-negative bacteria *in vitro* and in a systemic *Acinetobacter baumannii* mouse infection model. Importantly, this activity was retained in colistin-resistant strains.[189,190] Since pentamidine has already been in clinical use for the treatment of trypanosomiasis, leishmaniasis, and babesiosis since 1937, its safety and side effects have been extensively assessed. Recent efforts have yielded lipophilic vancomycin analogues that were able to permeabilize both the inner and outer membrane of Gram-negative bacteria while retaining their ability to inhibit peptidoglycan activity.[191]

Attempts to fuse outer membrane-penetrating peptide sequences to the lantibiotic nisin, which by itself is not active against Gram-negative bacteria due to its inability to reach its inner membrane target, have resulted in hybrid molecules with significantly increased anti-Gram-negative activity.[192,193] A similar approach is the hybridization of antibiotics with the aminoglycoside tobramycin.[194] Tobramycin is known as a ribosome inhibitor but at higher concentrations primarily attacks and permeabilizes the outer membrane.[195,196] It has been conjugated with efflux pump inhibitors,[197] quinolones,[198,199] and the chelator cyclam.[200] The resulting conjugates turned out to be potent antibiotic adjuvants and increased the activity of tetracyclines, fluoroquinolones, and β-lactams against Gram-negative bacteria by increasing uptake and limiting efflux. This is achieved by a multifaceted mechanism involving outer membrane permeabilization, impairment of efflux pumps, and depolarization of the inner membrane.[117,194,197−200] Similar results have been obtained for tobramycin dimers.[201]

Another very interesting example are short peptide sequences derived from the cell cycle proteins FtsA and MreB from *E. coli*. These proteins bind to the cytoplasmic membrane with an amphipathic α helix motif. Peptides derived from these membrane-anchoring sequences not only display potent activity against *E. coli* and other Gram-negative bacteria but also significantly increase their outer membrane permeability.[186,202,203]

Few compounds are known that selectively permeabilize the outer membrane, and most known compounds also permeabilize the inner membrane. Such dual membrane activity can often be a sign for poor selectivity for bacteria, and side effects are likely. This is, for example, the case for colistin and polymyxin B and the reason why they are only employed as the last resort antibiotics for otherwise resistant infections.[98,204−206] However, colistin nonapeptide and polymyxin B hepta-, octa-, and nonapeptide are derivatives of these compounds that retain their membrane-permeabilizing but not antibacterial activity, indicating high selectivity.[184] Such derivatives, particularly polymyxin B nonapeptide, have been shown to act as strong potentiators of antibiotics with

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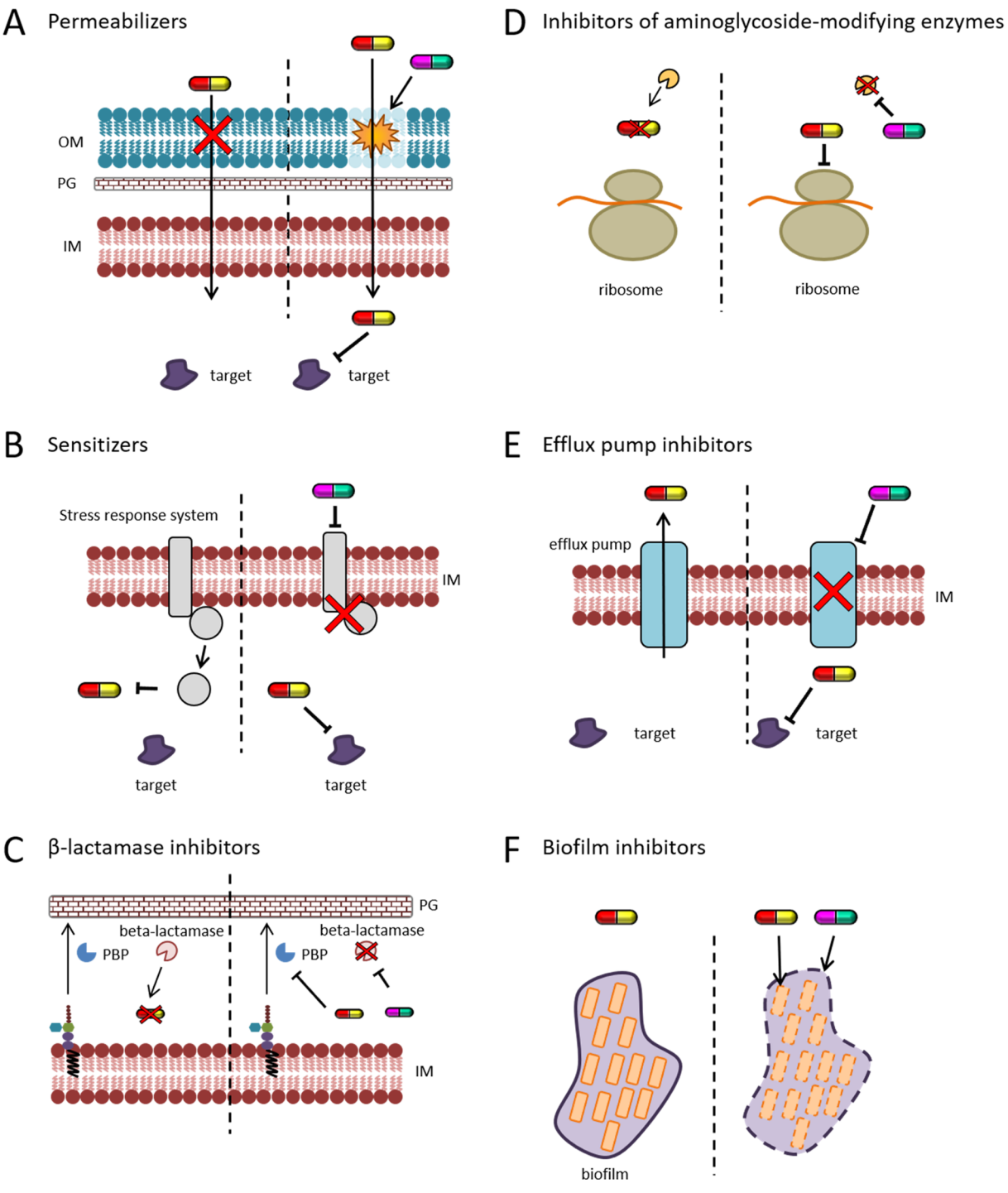

Figure 4. Mechanisms of resistance-breaking and antibiotic-potentiating compounds. (A) Cell envelope permeabilizers, (B) antibiotic sensitizers, (C) β-lactamase inhibitors, (D) inhibitors of aminoglycoside-modifying enzymes, (E) efflux pump inhibitors, and (F) biofilm inhibitors. red−yellow: antibiotic; magenta−turquoise: potentiator.

otherwise poor anti-Gram-negative activity.[184,207,208] At the same time, it is much less toxic than full-length polymyxin B.[184,209,210] While these properties have been demonstrated by many different studies, it is not used for combination therapy in the clinic.[211] However, a polymyxin derivative with similar properties as polymyxin B nonapeptide, NAB741, has been developed and successfully passed its first phase I clinical trial.[211] Compounds like polymyxin B nonapeptide or NAB741 hold great promise as potentiators of a wide range of antibiotics that would otherwise be ineffective against Gram-negative infections. It remains exciting to see how this type of compound proceeds in future clinical trials.

Sensitizers. A different strategy to enhance the performance of existing antibiotic drugs is to sensitize bacteria to their action. This can be achieved by inhibiting bacterial stress response systems that allow bacteria to adapt and survive antibiotic exposure (Figure 4B). Many studies on bacterial stress responses have proposed this as an option for future drug development, yet this has almost never been pursued. One exception is the bacterial SOS response to DNA damage.

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Table 4. Examples for Resistance-Breaking Compounds

resistance breaker	antibiotic	mechanism	ref
clavulanate[a]	amoxicilline	β-lactamase inhibitor	165, 166
avibactam[a]	ceftazidime, ceftaroline, aztreonam	β-lactamase inhibitor	221
vaborbactam[a]	Meropenem	β-lactamase inhibitor	221
tazobactam[a]	ceftolozane	β-lactamase inhibitor	221
compound 1	amikacin	inhibitor of aminoglycoside-modifying enzymes	222
PAβN	erythromycin, chloramphenicol	efflux pump inhibitor	223
verapamil	bedaquiline, ofloxacin	efflux pump inhibitor	224, 225
IMP-1700	quinolones	sensitizer (SOS response)	212, 217
colistin[a]	rifampin	permeabilizer	226−228
dispersin B	several possible	biofilm inhibitor	229−231
dehydrocrepenyc acid	several possible	inhibitor of horizontal gene transfer	232
streptazolin	several possible	immunomodulator	233

[a]Combination in clinical use.

DNA damage is sensed by the RecA protein, which induces autoproteolysis of the LexA repressor, leading to derepression of DNA repair genes. It has been shown that genetically impairing this stress response mechanism leads to reversion of quinolone resistance in *E. coli*.[212] Few inhibitors of DNA repair have been verified so far. For example, *p*-coumaric acid was found to interfere with the DNA-binding ability of RecA in *Listeria monocytogenes* and increased the activity of ciprofloxacin against this pathogen.[213] Another study found that zinc inhibits the ability of RecA to bind to single-stranded DNA, suggesting a potential application for zinc ionophores.[214] However, the effectivity and cytotoxicity of zinc ionophores would depend on the external zinc concentration,[215] which may limit this approach to topical applications or targeted drug delivery and release approaches. A large-scale screen for inhibitors of LexA autoproteolysis has yielded promising lead structures for the inhibition of this step of SOS response activation.[216] Similarly, IMP-1700, a rationally designed inhibitor of DNA repair, was later verified to target the AddAB DNA repair complex and sensitized multidrug-resistant *S. aureus* to ciprofloxacin.[217]

Another example for this strategy was identified by a transposon screen for increased tobramycin sensitivity in *Pseudomonas aeruginosa*. This screen identified the two-component stress response system AmgRS as a potential target for combination therapy with aminoglycoside antibiotics.[218] It was later found that the RNA polymerase inhibitor rifampicin was also a potent inhibitor of this stress response system and potentiated the activity of aminoglycosides like tobramycin, amikacin, gentamycin, and neomycin against *P. aeruginosa*.[219,220]

A multitude of stress response systems have been identified that play a role for antibiotic adaptation and resistance, yet little effort has been put into identifying inhibitors of these systems to be used as antibiotic potentiators. In view of the urgent need for novel antibacterial strategies, this possibility deserves more attention.

Resistance Breakers. A similar strategy, yet much more exploited, is the direct targeting of bacterial resistance mechanisms (Table 4).

The most prominent example for this is the inhibition of β-lactamase activity[234] (Figure 4C). Having pioneered the field, it was the first resistance-breaking strategy to be applied in the clinic. It is also the only combination of antibiotic and potentiator in current clinical use apart from synergistic combinations with the outer membrane-permeabilizing peptides colistin and polymyxin B. The oldest combination of β-lactam antibiotic and β-lactamase inhibitor is amoxicillin−clavulanate, which was approved for clinical use in 1981. It is still in use today and remains the only one that is orally available.[235] Since then, several other β-lactamase inhibitors have been identified, and eight combinations are currently on the market: amoxicillin−clavulanate, ticarcillin−clavulanate, ampicillin−sulbactam, cefoperazone−sulbactam, piperacillin−tazobactam, ceftolozane−tazobactam, ceftazidime−avibactam, and Meropenem−vaborbactam.[221,235] Several other combinations are in different stages of clinical development.[221]

A similar strategy is the inhibition of aminoglycoside-modifying enzymes, which are responsible for the majority of cases of aminoglycoside resistance (Figure 4D). Different enzymes have been described that acetylate different sites of aminoglycoside antibiotics,[236] some of which are inhibited by Cu^{2+}, Zn^{2+}, and Cd^{2+} ions.[237] Inhibitors of these enzymes have been identified by molecular docking studies with virtual compound libraries and by screening libraries for *in vitro* enzyme inhibition.[222,238,239] One of these has been confirmed to restore the activity of amikacin against a resistant *Acinetobacter baumannii* strain.[222] However, none of these compounds is close to clinical development at the present time.

The most important intrinsic resistance mechanism, next to the outer membrane permeability barrier, is constituted by antibiotic efflux pumps. Particularly, Gram-negative bacteria possess different multidrug efflux pumps that can export a wide variety of antibiotics and antimicrobial molecules. The most prominent efflux pump type is the Gram-negative-specific resistance-nodulation-division (RND) superfamily, in particular the well-characterized AcrAB-TolC pump.[240] A wide variety of antibiotics can be the substrate of these export systems, and their overexpression leads to high level drug resistance.[240] This makes efflux pumps one of the biggest challenges in overcoming antibiotic resistance and at the same time an attractive target for resistance-breaking antibiotic potentiators (Figure 4E). Since the discovery of the very first efflux pump inhibitors reserpine and verapamil against the *S. aureus* efflux pump NorA in 1991,[240] a multitude of inhibitors have been identified for both Gram-positive and Gram-negative efflux pumps. Many of them have been found to increase the activity of drugs, which are normally ineffective due to active export. These molecules have been extensively reviewed elsewhere.[240−244] Despite these efforts, no efflux pump inhibitor has advanced to clinical development yet.

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Common limitations of these molecules appear to be a high toxicity for mammalian cells, low *in vivo* efficacy, and insufficient spectrum coverage.[240,245] More research into this topic is needed to fully understand how multidrug efflux pumps work and how they synergize with other resistance mechanisms, such as the outer membrane, to go forward with effective inhibitor design.

Prevention of Horizontal Gene Transfer. A very different strategy to target antibiotic resistance is inhibiting horizontal gene transfer. Target mutations are only one way for bacteria to become antibiotic resistant. The much more threatening possibility is the spread of resistance genes that are encoded on plasmids or other mobile genetic elements within a bacterial population. This is especially critical for otherwise slowly occurring resistance mechanisms like enzymatic resistance.[246] Importantly, the human (or animal) microbiota can act as a reservoir for resistance plasmids that may spread to pathogenic bacteria during infection. Thus, the administration of an inhibitor of horizontal gene transfer in combination with antibiotic treatment could decrease the incidence of resistance transfer from a reservoir to an infectious strain.[247] This is an interesting concept but remains to be tested in infection experiments. Several compounds have been identified that inhibit horizontal gene transfer, yet in the majority of cases, this activity turned out to be due to secondary effects.[248] However, a small number of specific inhibitors have been found. For example, unsaturated fatty acid species from tropical fruits and their synthetic derivatives were able to inhibit the transfer of the most important resistance-related plasmid types in several Gram-negative pathogens.[232,249] Likewise, a mutation of a competence-stimulating peptide from *Streptococcus pneumoniae* has resulted in peptide versions that reduced competence and, thus, horizontal gene transfer.[250] As mentioned earlier, zinc was able to inhibit the bacterial SOS response, which interestingly, also impaired horizontal gene transfer between enterobacteria.[214] Secretion systems could be a promising target for such molecules as well. Thus, the inhibition of type IV secretion, which is involved in conjugative DNA transfer, has been shown to be inhibited by small peptidomimetic compounds.[251] While more research is needed to verify the feasibility of these approaches in clinical settings, it is certainly an interesting new combination therapy strategy that deserves further exploration.

Biofilm Inhibitors. One big problem in the clinic is the production of biofilms by bacteria like *S. aureus* and *P. aeruginosa*, and biofilm inhibitors are potent potentiators for antibiotics used against such infections (Figure 4F). Strategies to prevent biofilm formation or disperse mature biofilms can commonly be divided into three categories: the inhibition of adhesion or extracellular matrix production, the inhibition of quorum sensing, and the dispersion of the extracellular matrix.[252] Several antimicrobial peptides, for example, LL-37, prevent adhesion of bacterial cells by inhibiting the initiation of biofilm production.[253] Furanones are structurally similar to the quorum sensing molecule *N*-acyl-homoserine-lactone and are thought to competitively inhibit binding to their cognate transcriptional regulator.[253] Several other analogues of quorum sensing molecules have been patented, yet studies on their clinical potential are yet to come.[254] The most prominent biofilm-dispersing agent is the glycoside hydrolase dispersin B, which directly targets the extracellular matrix.[230] Further small molecule inhibitors have been identified for all these mechanisms.[254−257]

Biofilm inhibitors are particularly relevant in the context of medical devices like catheters and of chronic lung infections, with *P. aeruginosa* being the most problematic pathogen due to its high intrinsic antibiotic resistance and the emergence of totally drug-resistant isolates.[258] Several compounds have been described that could be used against *P. aeruginosa* biofilms. Interestingly, biofilm-degrading enzymes have been shown to remain active when immobilized on a surface, suggesting future applications in medical device technologies.[259] Short glycans have been shown to disrupt *P. aeruginosa* biofilms and subsequently increase the efficacy of antibiotics against it.[260] The small peptide 1018 targets the stringent response (p)ppGpp signaling, which is involved in biofilm formation, and acts as a potent biofilm-dispersing agent not only for *P. aeruginosa* but also for other pathogens including *Klebsiella pneumoniae* and *S. aureus*.[261] Peptide 1018 also resulted in decreased virulence of *P. aeruginosa* in a murine skin infection model.[262] Similarly, small molecule quorum sensing inhibitors have been shown to increase the efficacy of tobramycin against *P. aeruginosa* in a foreign-body infection model in mice.[263] Micafungin, which inhibits the synthesis of the pseudomonal cell wall component 1,3-β-D-glucan, was demonstrated to prevent *P. aeruginosa* biofilm formation and improved the outcome of antibiotic therapy in *P. aeruginosa*-infected mice, particularly when combined with the fluoroquinolone levofloxacin.[264] Interestingly, when the fatty acid synthesis inhibitor triclosan, which has been commonly used in hygiene products like toothpaste, was applied together with aminoglycosides like tobramycin, a strong antibiofilm activity was observed. Neither of these compounds have an effect on biofilms by themselves, and the mechanism behind this adjuvant activity is unknown.[265] So far, no biofilm inhibitor has made the transition to the clinic, yet a number of preclinical studies show the promise of using such agents as antibiotic potentiators in the future.

Multidrug Approaches. Combination therapy approaches may involve more than two compounds. This is, for example, very common in tuberculosis treatment regimes, which often consist of a combination of rifampicin, isoniazid, ethambutol, and pyrazinamide[266] but has also been employed for other bacterial infections.[267] A newly emerging approach is the combination of an antibiotic with more than one potentiator. This has, for example, been explored for the combination of β-lactams with β-lactamase inhibitors and permeabilizers such as dimeric tobramycin and tobramycin−cyclam.[200,201] However, such combinations are not currently used in the clinic.

Challenges of Combination Therapy. To date, antibiotic combination therapy is rather a last resort for severe infections than a standard therapy option. It is normally only employed for infections with multiresistant bacteria that cannot be treated with monotherapy, for latent infections like tuberculosis, or for antibiotics that have a high risk of encountering resistance when applied alone, such as trimethoprim−sulfonamide or β-lactam−β-lactamase combinations. Just as multitarget antibiotics are more effective than single-target antibiotics,[267] combination therapy is superior over monotherapy in terms of patient outcomes, at least when it comes to infections with multiresistant pathogens.[268] The current developments on antibiotic adjuvants have shown great promise for new combination strategies being added to our clinically available repertoire soon. However, combination therapies also face challenges.

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One important limitation is the development of clinically useful formulations and treatment regimes. Finding the right dosing, treatment intervals, and total treatment duration can already be challenging for single drugs and gets much more complicated with each additional drug added to the regime, since each individual drug has its own pharmacodynamic and -kinetic properties and its own side effects. Matching these to elicit the desired outcome in patients can be a serious challenge.[267,269] A solution for this limitation could be antibiotic hybrids as discussed in Designer Dual-Target Compounds. However, hybrid molecules may encounter other challenges like solubility and uptake.[125]

Another risk of combination therapy is antimicrobial resistance. While many empirical studies have found that drug combinations suppress resistance,[198,269−276] other studies have warned that under certain conditions antibiotic combinations might even promote faster resistance development. This is a particular concern for synergistic drug combinations. While they allow one to lower the dose and thus reduce side effects,[277,278] they come with an inherent risk of treatment failure through resistance: If resistance to one drug occurs, synergistic effects are lost, resulting in the low-dose exposure to a single antibiotic.[279−283] Even with nonsynergistic antibiotic combinations, the risk for increased resistance development is evident. A recent study found that the development of tolerance, i.e., the ability of bacteria to survive longer under antibiotic exposure without changing the antibiotic's minimal inhibitory concentration, to one drug may promote the transmission of resistance to the second drug.[284] These unwanted effects of combination treatments seem to be due to the prolonged survival of bacteria and their exposure to sublethal antibiotic concentrations. Therefore, the concept of "hitting them fast and hard", which has been proposed to be a key to successful antibiotic treatment, should be paid particular attention in combination regimes, even if lowering drug doses can be tempting to reduce adverse effects.[6,276]

CONCLUSION

Many fascinating approaches have been and are currently being developed to target multiple molecules in bacterial cells. From antibiotics that have multiple downstream effects, true multitargeting compounds, and dual-activity hybrid molecules to combination approaches with antibiotic adjuvants, all of these strategies have shown a certain promise and give hope for one to see light at the end of the tunnel of antibiotic development. Recent research gives the concept of polypharmacology another dimension by designing antibiotic adjuvants that possess multiple mechanisms themselves, such as outer membrane permeabilization and efflux pump inhibition, which synergistically increase the intracellular antibiotic concentration.[197] While more research is needed to truly understand the complex mechanisms underlying some of these multiple activities, they have already taught us one important thing, namely, that we should not shy away from complex mechanisms and multiple targets but try to exploit their full potential for future drug development.

AUTHOR INFORMATION

Corresponding Author

Michaela Wenzel − *Division of Chemical Biology, Department of Biology and Biological Engineering, Chalmers University of Technology, 412 96 Gothenburg, Sweden;* orcid.org/0000-0001-9969-6113; Phone: 0046-31-772-2074; Email: wenzelm@chalmers.se

Author

Declan Alan Gray − *Newcastle University Biosciences Institute, Newcastle University, NE2 4HH Newcastle upon Tyne, United Kingdom*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsinfecdis.0c00001

Author Contributions

Conceptualization, writing, and visualization: D.A.G. and M.W.

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