ANTIMICROBIAL RESISTANCE

Drug combinations: a strategy to extend the life of antibiotics in the 21st century

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Abstract | Antimicrobial resistance threatens a resurgence of life-threatening bacterial infections and the potential demise of many aspects of modern medicine. Despite intensive drug discovery efforts, no new classes of antibiotics have been developed into new medicines for decades, in large part owing to the stringent chemical, biological and pharmacological requisites for effective antibiotic drugs. Combinations of antibiotics and of antibiotics with non-antibiotic activity-enhancing compounds offer a productive strategy to address the widespread emergence of antibiotic-resistant strains. In this Review, we outline a theoretical and practical framework for the development of effective antibiotic combinations.

Antibiotics are unique among drugs for two principal reasons: they must meet particular and demanding criteria for efficacy and safety and they are highly susceptible to startlingly rapid loss of effectiveness through bacterial evolution by natural selection (BOX 1). These parameters have directed antibacterial drug development since the launch of the modern antibiotic era over six decades ago. As a direct evolutionary consequence of widespread antibiotic use and overuse, complicated by a retreat of a substantial portion of the traditional pharmaceutical industry from the field, the current limited extent of antibiotic drug development is being massively outpaced by emerging resistance against all available antibiotics[1]. Given this growing crisis, it is imperative that we critically examine our accepted drug discovery and development strategies and ask where we can innovate to address the gap between the availability of new antibiotic drugs and the growing need to combat antimicrobial resistance[2].

Retrospective analyses of antibiotic discovery and their implementation as drugs reveal that agents directed against a single protein target are less successful than those directed against multiple molecular targets or against multisubunit macromolecular machines or structures[3]. The latter often comprise intricately connected, redundantly encoded subunits that cannot be easily altered by mutation in a single gene. For example, resistance quickly arose to the synthetic sulfonamide drugs, the first broadly effective antibiotics discovered in the 1930s[4]. We now know that these compounds target a single essential metabolic enzyme, dihydropteroate synthase, and that resistance principally arises either by point mutations in the target, which is encoded by a single chromosomal gene[5,6], or by the lateral gene transfer of insensitive alleles[7,8]. By contrast, natural-product antibiotics such as β-lactams, aminoglycosides and tetracyclines — discovered a decade after the sulfonamides — were much slower to succumb to resistance by genetic mutation in the molecular targets. Penicillins inactivate not one, but a constellation of critical enzymes, the penicillin-binding proteins (PBPs) encoded by many different genes, required for cell wall synthesis, and this inactivation initiates a cascade of downstream effects leading to cell death. Similarly, tetracyclines and aminoglycosides target the bacterial ribosome, which consists of ~50 proteins and three large ribosomal RNAs (rRNAs) generally encoded in multiple gene copies on the bacterial chromosome. On the basis of this history and more recent experience from campaigns in the pharmaceutical industry focusing on single-agent (monotherapy) compounds that narrowly target essential enzymes, Lynne Silver has convincingly argued that we should consider an additional criterion in antibiotic drug discovery — the need for drugs to engage multiple cellular targets[3]. Indeed, most of the clinically successful antibiotic drugs fall into this category. More generally, effective drugs against cancer and other diseases often unexpectedly inhibit multiple targets in parallel, a concept referred to as polypharmacology[9,10].

This analysis predicts that multitarget engagement in antibiotic action should emerge as a frequent property of antimicrobial natural microbial products, which are optimized by millions of years of evolution to improve competitive fitness of the producer organism. We have benefited from this ancient history embedded in the genomic information of millions of bacterial and

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Box 1 | **General principles of antibiotic drug development**

Four key attributes define the ideal antibiotic drug. First, it must have little or no impact on human biochemistry to minimize toxicity during treatment. Thus, antibiotics must be exquisitely tailored towards vital microbial processes, preferably exclusive to pathogens while sparing benign components of the microbiome. Second, to combat what is often aggressive growth of microorganisms during infection, there is a need to achieve sufficiently high growth inhibitory concentrations over a short dosing period. Achieving these high growth inhibitory concentrations usually requires much higher quantities of antibiotic (grams to attain micromolar concentrations) than are typical for drugs that target human biology (milligrams to achieve nanomolar concentrations). Third, antibiotic drugs must reach a level of potency that most other drugs do not have to meet. That is, it is insufficient to merely attenuate microbial growth; a successful antibiotic must rapidly and efficiently entirely arrest growth. By contrast, the clinical efficacy of other drugs such as cholesterol-lowering agents or blood pressure medicines is achieved without the need for full inhibition of the physiological target. Fourth, successful drugs must have physicochemical properties that enable them to access and penetrate not only infected host tissues but also the bacterial pathogens themselves, all the while maintaining their growth-inhibiting activity. These attributes of successful antibiotic drugs present formidable objectives to meet in drug discovery and development and contribute to the challenge of new antibiotic development[1,147].

The second principal reason for the uniqueness of antibiotics in comparison to other drug classes is that antibiotics are susceptible to powerful and widespread evolutionary forces. Successful antibiotics must, therefore, be immune to random variation that occurs during DNA replication of pathogenic bacteria, changes that can occur so rapidly as to emerge over the course of infection treatment. In practice, the frequency of resistance must be $<10^{-8}$ at four times the minimal inhibitory concentration (MIC) to be considered a viable drug candidate during development. Furthermore, most bacteria undergo lateral gene transfer, which offers a broad conduit to achieve resistance by acquiring genes from other organisms. Good antibiotic candidates, although never wholly impervious to the development of resistance, must be minimally vulnerable to this problem.

Finally, an emerging property of successful antibiotic drugs is the ability to engage multiple physiological targets (see the main text). This ability includes members of redundant but distinct enzymes such as the penicillin-binding proteins as well as the products of genes that are frequently found in many copies such as components of the ribosome.

fungal species by harnessing these compounds as the foundation of antibiotic therapy for the past six decades[11]. By contrast, it has proved difficult to engineer de novo multitarget engagement in single synthetic compounds, a fact that we argue has contributed to the poor record of synthetic compounds in antibiotic discovery and development[12,13]. This experience predicts that a return to natural products as sources of leads for new antibiotics should be prioritized[14]. That said, identifying novel candidate multitarget compounds using traditional growth inhibition phenotype screens is plagued by the re-discovery of known antibiotic scaffolds. This challenge, termed dereplication, makes this strategy risky and in need of innovation to improve outcomes. Approaches such as post-assay dereplication using resistance profiles[15] or mass-spectrometry-based metabolomic fingerprinting[16,17] are used with some success, but these triage steps remain resource-intensive.

Although engineering multiple target engagement in single compound design and synthesis may be impractical, it can nevertheless in principle be achieved through the combination of distinct compounds. Bacteria and fungi are the most prodigious producers of selective antimicrobial substances, and yet these organisms rarely produce single compounds but rather generate complex mixtures of products that interact to achieve complementarity and synergy[18]. Similarly, plants and insects produce a multitude of nonspecific compounds that collectively combat infectious pathogens[14,19]. In the instances when combination strategies have been systematically pursued in the clinic, therapeutic success has been attained for HIV[20], cancer[21], cardiovascular disease[22] and many others, including antibacterial strategies, as discussed below.

The historical success of monotherapy using (mostly) natural-product antibiotics to treat bacterial infections has, with a few exceptions, been regarded as a mandatory criterion in new drug discovery. Drug developers rightly prefer to focus expensive clinical trials on a single, well-characterized, distinct compound, and for dosing simplicity, clinicians favour single-agent drugs over more complex mixtures. However, with the realization that successful single-agent antibiotics are engaging many targets and that compounds with a single molecular target have substantial resistance liabilities, the focus on monotherapy needs to be revisited.

As we continue to understand the systems biology of antimicrobial action (that is, the chemical–genetic interaction networks specific to antibiotics), Silver's observation a decade ago that the most successful antibiotic drugs engage multiple targets[3,23] now has the potential to guide knowledge-based antibiotic combination drug discovery. Currently, the combination of individual antibiotics seeks to meet several objectives: first, to increase the spectrum of antimicrobial coverage during empirical therapy when the identity of the pathogen is not known; second, to achieve synergistic effects and thus improve efficacy; third, to suppress the emergence of resistance; and fourth, to minimize host toxicity[24]. Indeed, in addition to multiple target engagement, one of the more important consequences of the combination of antibiotics, in particular at concentrations above the minimal inhibitory concentration (MIC), is the suppression of resistance.

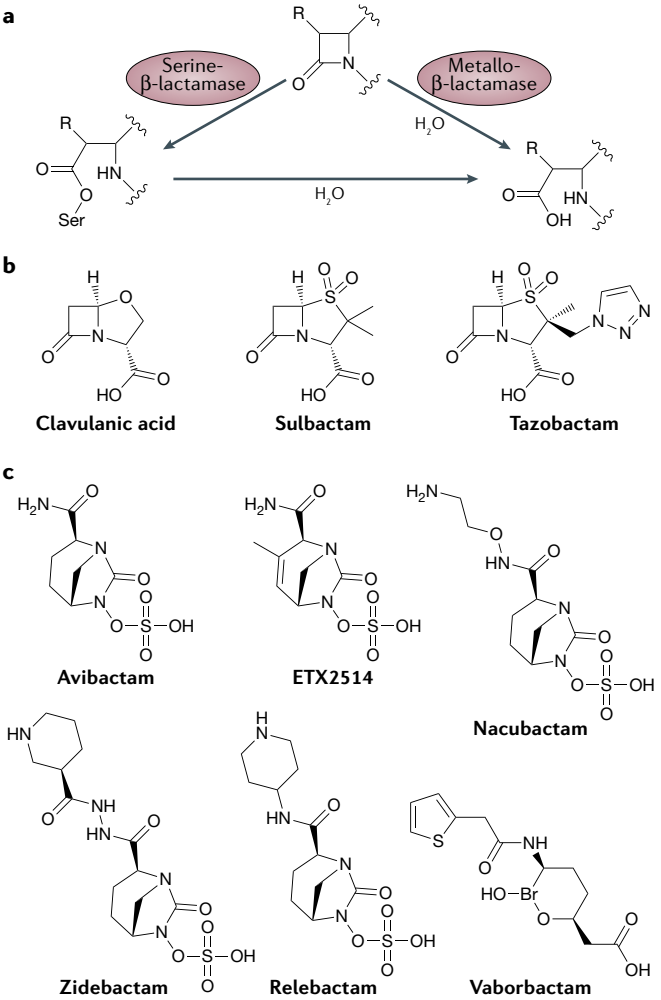
Soon after the many fundamental discoveries in the golden era of antibiotics (~1940–1960), attempts to combine these new drugs became commonplace. Many of these early combinations, generally of two antibiotics but also higher-order combinations, were assembled in an ad hoc manner with little rigorous understanding of drug efficacy or molecular mechanism[25]. In fact, many combinations may have been opportunistic attempts to establish patentable medicines because several early antibiotics were explicitly developed without patent exclusivity[26]. By the mid-1950s, >60 drug combinations were available, ranging from combinations of 2–5 different antibiotic and non-antibiotic components[26].

Recognizing that no antibiotic compound is universally efficacious for all infections, one of the first important drivers in combining antibiotics was the opportunity to provide superior efficacy over individual compounds. For example, the combination of streptomycin with penicillin was reported in 1950 (REF.[27]) and trimethoprim with sulfonamides in 1968 (REF.[28]); both combinations improved efficacy and antibacterial spectrum. In the treatment of tuberculosis, the combination of antibiotics to suppress the selection for resistance to single agents was recognized early in

Box 2 | **Syncretic β-lactam antibiotic–β-lactamase inhibitor pairs**

Resistance to the β-lactam antibiotics (penicillins, cephalosporins and carbapenems) in pathogenic bacteria occurs most commonly through the production of β-lactamases that hydrolyse the β-lactam ring of these antibiotics that is essential to their antimicrobial activity[148]. β-Lactamases use one of two distinct chemical mechanisms to achieve β-lactam ring opening (see the figure, part **a**). The first mechanism uses an active-site Ser residue that forms a transient covalent bond with the antibiotic followed by hydrolysis of the enzyme-associated ester to generate the inactive antibiotic. The second mechanism achieves β-lactam hydrolysis through metal (usually two Zn^{2+} ions)-assisted activation of a water molecule to generate the hydrolytic species. Serine-β-lactamases such as TEM, SHV and CTX-M have historically been the dominant enzymes in pathogens, but in the past few years, metallo-β-lactamases (for example, NDM, VIM and IMP) have been increasingly problematic in the clinic.

In 1976, scientists at Beecham Pharmaceuticals reported on a new β-lactam compound with weak antibiotic activity but potent inhibition of serine-β-lactamases[149]. This molecule, clavulanic acid (see the figure, part **b**), was paired with amoxicillin in the first clinically syncretic β-lactam antibiotic–β-lactamase inhibitor combination called Augmentin. Augmentin was a clinical and financial success, spurring the discovery of the penicillin sulfones serine-β-lactamase inhibitors tazobactam and sulbactam in the 1980s and more recent advances[150] (see the figure, part **b**).



In 2016, the FDA approved the first member of a new class of serine-β-lactamase inhibitor, avibactam (see the figure, part **c**), a diazabicyclooctane with potent inhibition of many serine-β-lactamases that are poorly inhibited by existing inhibitors. A fixed-dose combination of avibactam with ceftazidime is sold under the name Avycaz. Several other diazabicyclooctanes are in various stages of development. In 2017, the FDA approved vaborbactam, a new cyclic boronate chemical scaffold with serine-β-lactamase inhibition. The combination of vaborbactam and meropenem is available under the trade name Vabomere. Despite the growing importance of metallo-β-lactamases in the clinical failure of β-lactam therapy, no inhibitors of these enzymes are currently in late-stage clinical development[62].

the 1950s[29], whereas the advantages of antibiotic combinations to treat leprosy were reported in the 1960s (but were not subject to clinical agreement until the 1980s)[30]. Such combinations, now backed by rigorous mechanistic, clinical and epidemiological data, remain in frontline use today. Nevertheless, with the exception of β-lactam–β-lactamase inhibitor combinations (BOX 2), formulated fixed-dose antibacterial drug combinations remain rare in drug formularies (fixed dose being desirable to ensure patient compliance in out-of-hospital use). However, empirical combinations of antibiotics to achieve coverage of pathogen spectrum and/or combat resistance remain common in clinical practice[24].

This fairly narrow application of antibacterial combinations contrasts with clinical practice in other areas of infectious disease such as antivirals, in which drug combinations are standard to suppress or overcome resistance[20]. In the current antibiotic crisis, bacterial pathogens are increasingly resistant to all available antibiotic drugs, often through redundant mechanisms and to multiple antibiotics in the same organism[31]. This situation has been exacerbated by the lack of innovation in the discovery of new agents that meet the stringent criteria for successful antibiotic drugs[1].

Given all of the above, it is time to reconsider the monotherapy standard and actively explore combination therapies to achieve multitarget engagement and diminish the emergence of spontaneous resistance. In this Review, we discuss the rationale of drug combinations to overcome resistance and improve the efficacy of antibiotics. We focus particularly on compounds that enhance the activities of antibiotics, termed antibiotic adjuvants, rather than combinations of existing antibiotics as this is well covered in other reviews[24,32].

We further explore the successes and challenges of these combinations in antibiotic drug development.

Synergy and antagonism

A common objective in combining bioactive compounds is to achieve synergy, a concept in drug action that is often misunderstood and requires careful definition[33]. Previously, a unifying nomenclature to describe antimicrobial combinations has been proposed that we adopt in this Review[34]. Congruous combinations of antibiotics are based on compounds that individually have cell growth inhibition activity towards the target organism (FIG. 1). This concept is the basis for most existing antibiotic combination therapies. By contrast, syncretic combinations include at least one component that does not have overt antibiotic activity (FIG. 1). The β-lactam–β-lactamase inhibitor pairs are the best examples of syncretic combinations in current clinical use (BOX 2). A third type of combination, termed coalism, occurs between compounds that alone have no antibiotic activity but that together are active (FIG. 1). The opposite of synergy is antagonism, whereby the sum of the activities of the individual components is diminished in the combination. As described below, each of these classes of drug–drug interaction can be described in terms of the underlying genetic interactions.

Synergy and antagonism are formally calculated in the microbiology laboratory through the fractional inhibitory concentration index (FICI)[35,36]. This approach takes advantage of the traditional determination of the MIC whereby cell growth is measured in liquid culture against a series of (generally twofold) dilutions of antibiotic. By systematically varying both antimicrobial compounds (A and B) in a checkerboard fashion, interactions that result in synergy, no interaction or antagonism are readily apparent (FIG. 2a). The fractional inhibitory concentration (FIC) is defined as the MIC of compound A in the presence of B divided by the MIC of A, whereas the FICI is the sum of the FIC of compounds A and B. In principle, then, an FICI of ≤1 would suggest synergy. However, because of the well-accepted limitations of the accuracy of the broth dilution method of one twofold dilution above and below the MIC, an FICI of ≤0.5 is required for synergy and a value of ≥4.0 is required for antagonism[36]. All other values are accepted to indicate no interaction. Frequently, follow-up time-kill studies are used to confirm synergism. In these studies, synergistic combinations should decrease the colony forming units concentration by a factor of at least 2 $\log_{10}$ per millilitre (REF.[35]).

The advantage of the FICI approach is its simplicity and speed. Nevertheless, it is a fairly crude measure of synergy that, although well suited to the high-volume clinical microbiology laboratory, does not provide finer dose-dependence information that is potentially available from an analysis of smaller drug intervals. This failure is due to the accepted twofold error in MIC, noted above, and the norm, established almost a century ago, of increasing antibiotic concentrations by a factor of 2 in each dilution (for example, a typical series would be 1, 2, 4, 8, 16, 32, 64, 128 μg ml^{-1}). Furthermore, this approach may obscure more subtle interactions that can inform the mechanism of action of compound pairs, especially in the early stages of discovery. Two general models of potential drug interactions are recognized: Loewe additivity, which states that the active compound cannot interact (positively or negatively) with itself; and Bliss independence, which assumes that two compounds do not interact with each other[33]. When deviance from these null hypotheses occurs, synergy or antagonism is implied. To distinguish synergy or antagonism from additivity, fitting to the general model (Loewe or Bliss) followed by graphical analysis using an isobologram is performed[37] (FIG. 2a). This approach enables finer measurement of drug–drug interactions beyond the standard checkerboard approach and can provide additional mechanistic information. In practice, the distinction between the Loewe and Bliss models is challenging in antibiotics research given the errors in MIC measurement, but the Loewe additivity model is more intuitive. Using such careful approaches, previous studies have uncovered important and often non-obvious antibiotic interactions[38,39]. These include the observations that antagonistic drug pairs can suppress resistance[40], that synergistic combinations can counterintuitively drive more rapid evolution of resistance than individual antibiotics[41], that alternating drug treatments can be more efficacious than co-treatment[42] and that higher-order combinations can lead to dose orthogonality that is difficult to model and can often result in unexpected interactions[43–46].

Antagonism is not uncommon in antibiotic combinations[24]. Antagonism can occur when one antibiotic inhibits the cell death mechanism of another. For example, bacteriostatic inhibitors of protein synthesis such as tetracyclines or chloramphenicol can prevent the synthesis of lytic autolysins that are required for the bactericidal activity of β-lactam antibiotics. Although antagonism is generally not considered desirable in a drug combination, as noted above, it was previously shown that hyper-antagonism (concentrations at which the drugs suppress

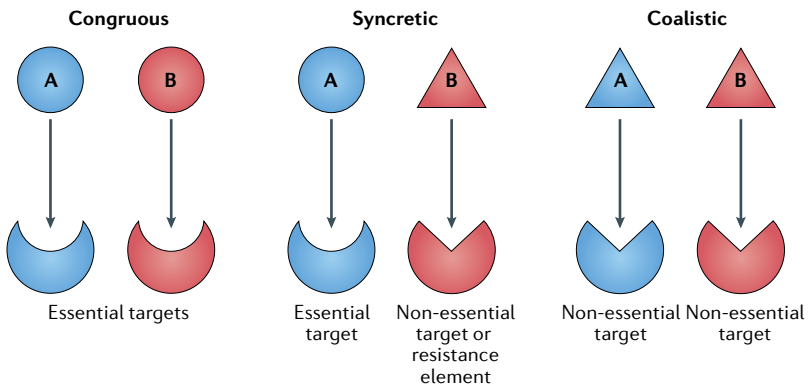


Fig. 1 | **Classification of synergistic antibiotic combinations.** In a congruous pair, two antibiotics (A and B) that target distinct essential molecular processes can display synergy. By contrast, syncretic combinations include an antibiotic (A) that targets an essential process and a non-antibiotic adjuvant (B), the molecular target of which is a resistance element (Class Ia) or a non-essential bacterial (Class Ib) or host (Class II) target. Coalistic pairs (A and B) are compounds without antibiotic activity that target non-essential but synthetically lethal gene functions.

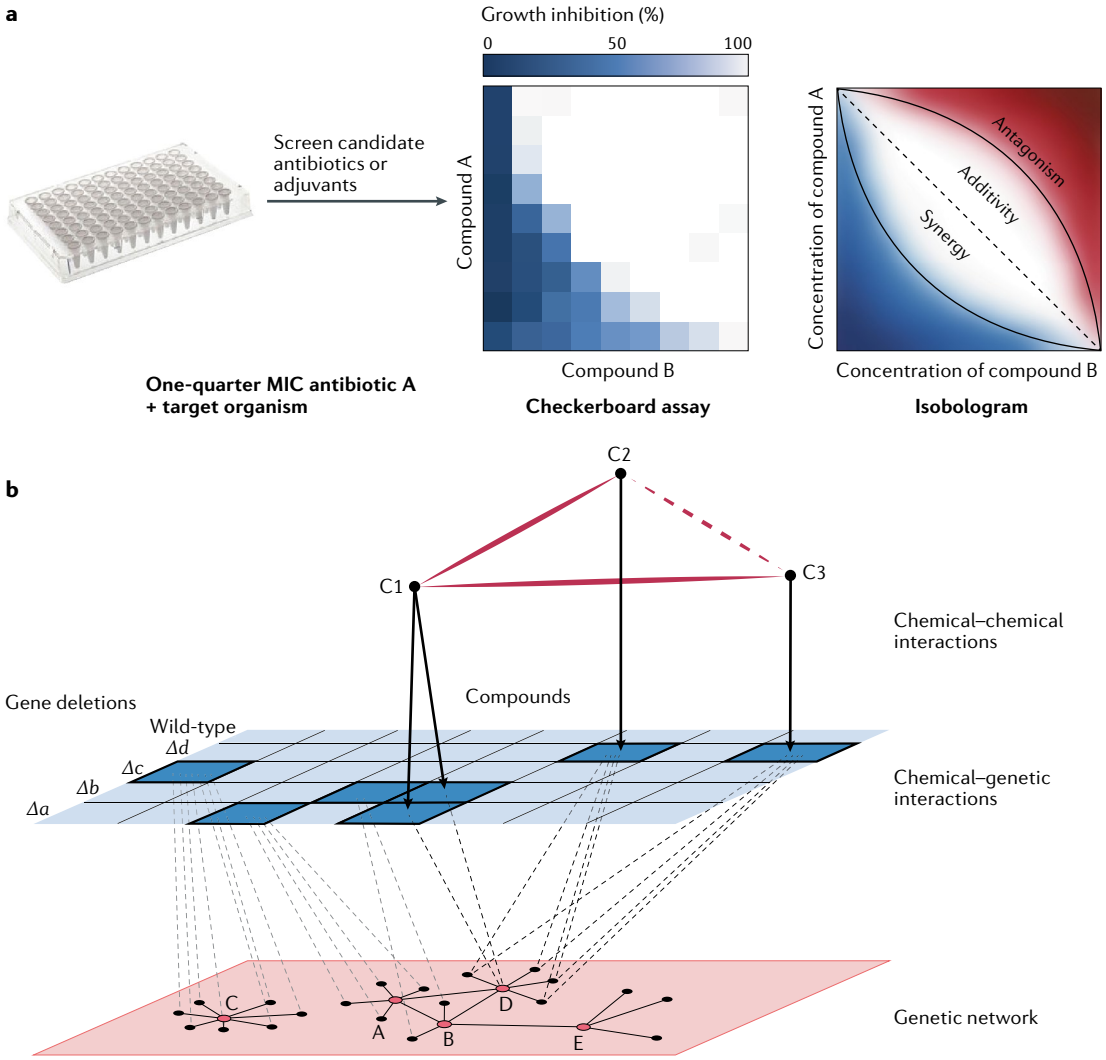


Fig. 2 | **Identifying synergistic antibiotic combinations. a** | Potential synergistic combinations can be identified in screening campaigns in which customarily a sublethal (one-quarter minimal inhibitory concentration (MIC)) concentration of an antibiotic (compound A) is used in the presence of a susceptible organism and candidate compounds (compound B) are systematically assayed for growth inhibition. Synergy can be confirmed using either a checkerboard array with determination of the fractional inhibitory concentration index (FICI) or a finer gradient isobologram approach fitting data to Loewe or Bliss models. **b** | The molecular basis of synergy lies in the vulnerability of genetic networks to pairwise combinations of chemical inhibitors. In the genetic network, nodes (A, B, C, D and E) represent genes and lines that connect nodes represent synthetic lethal interactions, also known as negative genetic interactions. This genetic architecture dictates synthetic lethal chemical–genetic interactions between chemical inhibitors and genetic mutations, which may be loss-of-function deletion strains in high-throughput screens (indicated by *Δa*, *Δb*, *Δc* and *Δd*) or conditional alleles of essential genes. Dark blue squares indicate chemical–gene interactions across the potential chemical–genetic space. In turn, chemical–chemical interactions are determined by the underlying genetic and chemical–genetic relationships. For example, a chemical interaction with *Δc* could be the result of inhibition of any gene linked to C by a synthetic lethal relationship, shown by dashed lines. In this example, compound C1 is synthetic lethal with *Δa* and *Δb*, concordant with D as the target of C1. Compounds C2 and C3 target other synthetic lethal interaction partners of D and hence are predicted to be synergistic with compound C1 (red line). An additional potential three-way combination of C1, C2 and C3 can be inferred from the genetic and chemical–genetic relationships (dashed red line). Similar arguments hold for suppressive genetic (positive) interactions and chemical antagonism. Part **b** is adapted with permission from REF.[135], Elsevier.

each other[38]) can be advantageous in certain drug pairs in the suppression of resistance by selectively advantaging antibiotic-sensitive strains over resistant ones at high concentrations of antagonistic drug concentrations[40]. Theoretically, under certain competitive conditions[47], such combinations could be used to selectively remove resistant strains from a given bacterial population.

Congruous antibiotic combinations

Congruous synergistic antibiotic combinations have proved historically effective — for example, the combination of penicillin with streptomycin for enterococcal infections and a combination of rifampin–isoniazid–pyrazinamide in the treatment of tuberculosis[24]. A recent study showed that such combinations can also

overcome acquired resistance in the case of Mcr1-mediated colistin resistance[48]. Nevertheless, there are currently few formulated fixed-dose antibiotic combinations. One example is co-trimoxazole (trimethoprim-sulfamethoxazole in a 1:5 weight/weight (w/w) ratio), which is sold under various trade names, including Septra and Bactrim. Synercid is a synergistic combination of streptogramin antibiotics comprising quinupristin and dalfopristin in a 3:7 w/w ratio[49]. Other commercially available antibiotic drug combinations include topical agents such as bacitracin and polymyxin B (Polysporin; sometimes with the addition of gramicidin) and Neosporin, which combines neomycin, bacitracin and gramicidin. These combinations are synergistic in some bacteria[50,51] and offer broad-spectrum coverage of both Gram-positive and Gram-negative pathogens.

The paucity of other fixed-dose combinations of existing antibiotic drugs reflects the fact that single agents are for the most part effective and useful on their own whereas combinations of these strategies used in clinical practice are guided by need and experience. However, there is great opportunity to use the example of co-trimoxazole and streptogramins as a guide to systematically screen combinations of natural-product antibiotic candidates that were not pursued vigorously in the past (it is estimated that >20,000 antibiotics are already known[52]) or the thousands of synthetic compounds from the various discovery and development campaigns run in the pharmaceutical sector over the past 25 years. A concerted effort to make available libraries of these compounds could result in breakthrough opportunities for new congruous combination drugs.

Syncretic combinations

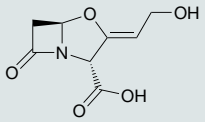
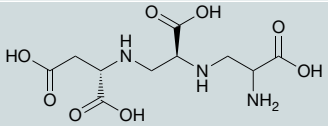
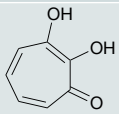
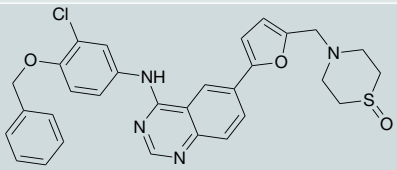
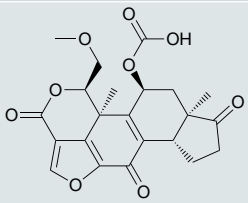
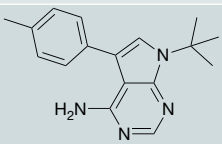
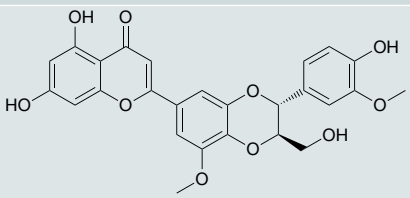
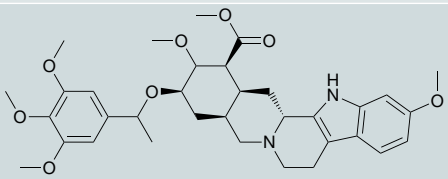
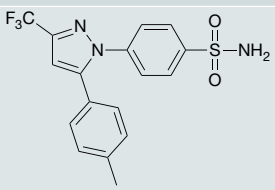
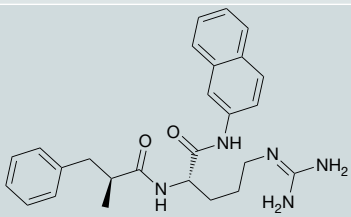
Compounds with little or no antibiotic activity but that enhance the efficacy of bona fide antibiotics in syncretic combinations are termed antibiotic adjuvants[53–56] or, more colloquially, resistance breakers[57]. Antibiotic adjuvants fall into two classes: Class I adjuvants act on bacterial metabolism or physiology, whereas Class II adjuvants increase antibiotic efficacy by altering host biology[56]. Class I adjuvants can further be differentiated into compounds that directly block resistance (Class Ia) (TABLE 1), exemplified by the β-lactamase inhibitor clavulanic acid discussed below and agents in BOX 2, and compounds that potentiate antibiotics through indirect mechanisms (Class Ib) (TABLE 2). The noteworthy advantage of adjuvants is that these offer a direct route to extend the life of existing antibiotic drugs that have proved so effective these past 60 years. Given the recognized difficulty in discovering and developing new antibiotics, reinvigorating old antibiotics with novel adjuvants is a feasible and cost-effective strategy.

Although combining antibiotics empirically to achieve coverage of a broad pathogen spectrum or in fixed-dose formulations to achieve synergy has often been successful, combining antibiotics with syncretic non-antibiotic bioactive compounds has met with mixed success. In the 1950s, the combination of antibiotics with a myriad of other compounds was commonplace but rarely rigorously shown to be effective. For example, achrocidin was a fixed-dose combination of five compounds (tetracycline (antibiotic), phenacetin and salicylamide (both analgesics with antipyretic activity), chlorothen (antihistamine) and caffeine (stimulant)) that was extensively marketed as a common cold medicine[58]. With the tightening of rules by the FDA that explicitly demanded evidence of improved efficacy in the 1960s, ad hoc efforts to devise syncretic combinations were largely abandoned. Drug combinations were revisited in the early 1980s with the discovery of the β-lactamase inhibitor clavulanic acid and its co-formulation with amoxicillin to generate the first highly effective antibiotic–non-antibiotic combination, called Augmentin, available in several fixed doses[59]. Clavulanic acid has little antimicrobial activity on its own but synergizes with amoxicillin in bacteria expressing susceptible β-lactamases. This discovery ushered in the development of several other β-lactam–β-lactamase inhibitor combinations that continue to be fruitful today (BOX 2). Of the antibiotic drug candidates currently in phase I, II or III stages of clinical development, 15% are syncretic β-lactam–β-lactamase inhibitor combinations (PEW Trust, retrieved January 2018). This general concept, sometimes termed an evolutionary trap[38,60], may enable other resistance determinants to be exploited as therapeutic targets.

Because syncretic antibiotic adjuvants do not have antibiotic activity themselves and thus lack an intrinsic MIC, quantitative assessment of their activity by traditional FICI determination is not possible. Instead, to quantify the effect of such adjuvants, one can determine a relative FICI using the highest concentration of adjuvant used; however, this must be explicitly stated to enable comparison between experiments. Fourfold lowering of the MIC of the antibiotic component is a standard requirement for synergy. A better measure of efficacy for Class I adjuvants is the rescue concentration, which is equivalent to the concentration of the non-antibiotic partner compound that lowers the MIC of the antibiotic component to the breakpoint (that is, the concentration of antibiotic that defines the border of clinical resistance versus susceptibility)[56]. For Class II adjuvants, a standard measure of efficacy has not yet been established, but a reasonable proposal, by analogy to the checkerboard synergy studies and syncretic Class I adjuvants, is a fourfold enhancement of antibacterial activity in a suitable cell or animal model. When whole-cell assays are available (for example, in the case of intracellular pathogens), the standard bacterial growth assays should be used.

Class Ia antibiotic adjuvants. Class Ia adjuvants are exemplified by the β-lactamase inhibitors (BOX 2). Direct blockade of enzyme-mediated drug resistance rescues the activity of the antibiotic and has proved to be clinically successful[59,61,62]. Other efforts to identify inhibitors of resistance include targeting aminoglycoside-inactivating enzymes[63–66] and ribosome methyltransferases that confer resistance to macrolide antibiotics[67–69], although none have thus far proved to be effective in animal models

Table 1 | **Examples of syncretic Class Ia antibiotic adjuvants**

Adjuvant	Structure	Mode of action	Refs
Clavulanic acid[a]		Serine-β-lactamase inhibitor	149
Aspergillomarasmine A		Metallo-β-lactamase inhibitor	151
7-Hydroxytropolone		Aminoglycoside adenyltransferase inhibitor	63
6-Furanylquinazolines		Aminoglycoside adenyltransferase (2′′)-Ia inhibitor	15
Wortmannin		Aminoglycoside kinase APH(2′′) inhibitor	152
Pyrazolopyrimidines		Aminoglycoside kinase APH(3′)-Ia inhibitors	65,66
5′-Methoxyhydnocarpin		Efflux inhibitor	74
Reserpine		Efflux inhibitor	153
Celecoxib		Efflux inhibitor	75
PAβN		Efflux inhibitor	76

APH, aminoglycoside phosphotransferase. [a]See BOX 2 for additional serine-β-lactamase inhibitors.

Table 2 | **Examples of syncretic Class Ib antibiotic adjuvants**

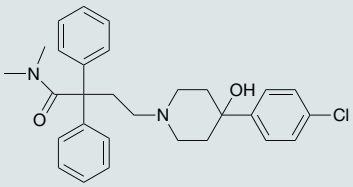
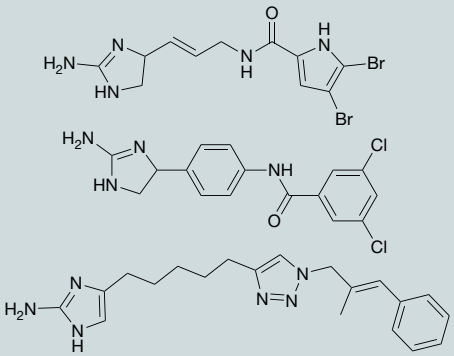
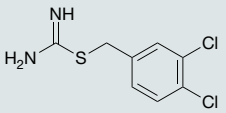
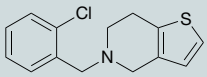
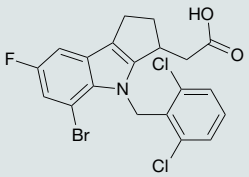
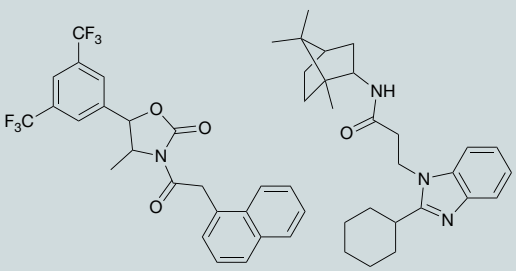
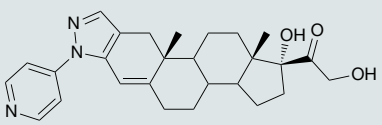
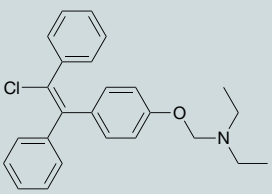
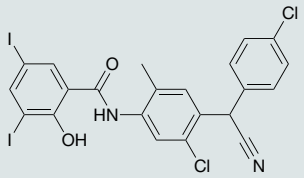
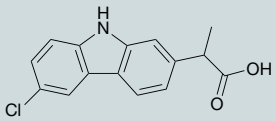
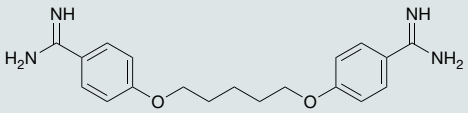
Adjuvant	Structure	Mode of action	Refs
Loperamide		Potentiates tetracycline antibiotics by perturbation of the cell membrane proton motive force	113
2-Aminoimidazoles		Potentiates antibiotic activity against drug-resistant pathogens	154–157
A22		MreB inhibitor that sensitizes *Escherichia coli* to novobiocin	158
Ticlopidine		Wall teichoic acid biosynthesis inhibitor synergizes with β-lactam antibiotics that are selective towards PBP2 in MRSA	159
Tricyclic indole acid L275		Wall teichoic acid biosynthesis inhibitor potentiates imipenem versus MRSA	160
Tarocins		Wall teichoic acid biosynthesis inhibitors (targeting TarO) synergize with β-lactam antibiotics against MRSA	161
Murgocil		MurG inhibitor potentiates imipenem against MRSA	162
Clomiphene		Inhibitor of undecaprenyl phosphate synthase synergizes with cell-wall-directed antibiotics in targeting MRSA	163

Table 2 (cont.) | **Examples of syncretic Class Ib antibiotic adjuvants**

Adjuvant	Structure	Mode of action	Refs
Closantel		Potentiates polymyxin B against *Acinetobacter baumannii*	164
Carprofen		Potentiates doxycycline against methicillin-resistant *Staphylococcus pseudintermedius*	165
Pentamidine		Lipopolysaccharide disrupter that potentiates antibiotics against Gram-positive bacteria (for example, rifampin and novobiocin) in *E. coli*	166

MRSA, methicillin-resistant *Staphylococcus aureus*; PBP, penicillin-binding protein; TarO, UDP-*N*-acetylglucosamine transferase responsible for the first step in wall teichoic acid synthesis.

of infection. A recent study described a platform whereby individual resistance elements are cloned into a uniform *Escherichia coli* host under control of constitutive strong (*bla*) and weak (*lac*) promoters[15]. This platform presents a streamlined screening and testing tool that improves traditional screening of drug-resistant pathogens, which often have poorly characterized genotypes and redundant resistance elements.

Another important target in the search for Class Ia adjuvants is antibiotic efflux (reviewed in REFS[70–72]). The cytoplasmic membrane-spanning major facilitator superfamily (MFS), found in both Gram-negative and Gram-positive pathogens, and the tripartiteresistance-nodulation-division (RND) systems, which span the inner cytoplasmic membrane, the periplasm and the outer membrane in Gram-negative bacteria, dominate as the major antibiotic resistance factors in the clinic[70]. Several efforts have been successful in identifying inhibitors of efflux in various bacterial species. For example, inhibitors of the *Staphylococcus aureus* MFS pump NorA include natural products from plants such as the alkaloid reserpine[73] and the flavonoid 5′-methoxyhydnocarpin D74 as well as synthetic compounds such as celecoxib and derivatives[75].

The central importance of RND-mediated efflux in Gram-negative bacteria has fuelled several campaigns to identify inhibitors. The canonical AcrAB–TolC system from *E. coli* has principally been the focus of these efforts given that it is the best structurally and functionally characterized system[70]. However, in the clinic, MexAB–OprM and MexXY–OprM of *Pseudomonas aeruginosa* and AdeABC of *Acinetobacter baumannii* contribute greatly to antibiotic failure during treatment. Inhibitors of the cytoplasmic membrane-spanning AcrB (equivalent to MexB, MexY and AdeB) have been identified and well characterized. Peptide analogues such as PAβN[76] and various synthetic small molecules, including aryl-piperazines[77] and pyranopyridnes[78], have been reported. However, none offer the hoped-for universal blockade of antibiotic efflux, likely because the structure of AcrB and analogous pumps suggests multiple substrate channels and efflux mechanisms[79]. PAβN also has off-target effects that complicate the assessment of efflux inhibition[80]. Recently, targeting of the periplasmic AcrA protein has been described, offering an alternative to AcrB inhibition[81].

Despite the attractiveness of directly targeting antibiotic efflux in the development of Class Ia antibiotic adjuvants, no clinical candidates have yet emerged. This failure reflects the substantial challenge in efficiently targeting this structurally complex target, especially in the case of multidrug resistance RND pumps that have evolved broad substrate specificity, the existence of redundant backup efflux systems present in most bacteria and the upregulation of expression of pumps as orthogonal contributors to resistance levels. Nevertheless, a pan-efflux inhibitor, even if species-specific, may yet be an achievable goal that would represent a breakthrough adjuvant perhaps through disruption of the cell membrane energization that is essential for pump activity.

Class Ib antibiotic adjuvants. Our understanding of cell biology has undergone a substantial paradigm shift since the first screens for antibiotics based on simple cell growth inhibition in the 1950s and 1960s and the subsequent focus on single-target-based biochemical screens as genome sequence information emerged in the 1990s and 2000s. The initial emphasis of antibiotic drug discovery in the ensuing post-genomic era was on targeting single, apparently essential, gene products (that is, those encoded by genes that, under various laboratory growth conditions, cannot be deleted). In virtually all species interrogated to date, the number of essential genes identified under laboratory conditions is <20% of the total gene complement[82], such that in bacteria the remaining 80% of genes are not seen as strong candidates for antibiotic discovery. However, extensive

drug discovery campaigns focused on essential gene products have not yielded any new antibiotics[12,13,83]. In contrast to this single-gene-centric view of biology, we now know that cell growth and physiology are not governed by a series of mostly independent and distinct metabolic pathways as we once thought and are often still taught in the biochemistry classroom. Instead, functional genomics approaches have revealed that gene functions are highly interconnected in redundantly networked systems that strongly buffer essential functions against the loss of any particular gene or its function. Pioneering systems genetics studies in yeast exploited genome-wide gene deletion collections to map the functional genetic interaction network of the cell by systematically assessing the phenotype of all possible double mutants[84]. At the same time, systematic proteomic studies revealed that most proteins in the cell participate in an extensively interconnected network that is nucleated on discrete protein complexes or interaction hubs[85–87]. The development of similar systems approaches in bacteria and other eukaryotic model systems has consolidated this network paradigm[88–91]. This physical and functional modularity of cellular organization in turn provides a rational framework for combination drug discovery[92,93].

The phenomenon of synthetic lethality, whereby the combination of two individually non-lethal mutations causes a lethal phenotype, was first discovered in the fruitfly *Drosophila melanogaster*[94,95]. The vast extent of synthetic lethal interactions, referred to as genetic buffering[96], became apparent only with the advent of systematic genetic array (SGA) screens in the budding yeast *Saccharomyces cerevisiae*, which exploited genome-wide collections of gene deletion strains[84]. A near-complete survey of all possible gene–gene interactions in yeast suggests that although only 1,000 genes are essential under laboratory growth conditions, >500,000 binary combinations of otherwise viable gene deletion alleles cause lethality[97]. Importantly, the buffering concept also applies to conditional or partial loss-of-function alleles of essential genes, and indeed such hypomorphic alleles tend to show fivefold more genetic interactions than deletion alleles of non-essential genes[98]. The combination of conditional alleles in essential genes, or of a conditional allele in an essential gene and a deletion of a non-essential gene, or of deletions in two non-essential genes, can therefore result in lethality. These genetic situations correspond to congruous, syncretic and coalistic drug pairs, respectively (FIG. 1). Genome-wide collections of engineered gene deletion strains in *E. coli*, termed the KEIO collection[99], and the fission yeast *Schizosaccharomyces pombe*[100] have enabled similar systematic genetic analyses in these species that also suggest that most genes exhibit a broad range of synthetic lethal interactions[82,88,89,101–103]. From the perspective of drug target space, these findings suggest that a much more substantial fraction of the genome can be targeted provided that two gene products can be simultaneously targeted. Moreover, the prevalence of genotype-specific lethalities between strain isolates further indicates that any given mutation may provide a contextual vulnerability that can be exploited for therapeutic benefit[104].

The genetic buffering concept underpins all forms of synergism but in particular provides a rational basis for the identification of non-antibiotic compounds that considerably enhance the activity of antibiotics (that is, Class Ib antibiotic adjuvants) (TABLE 2). Class Ib antibiotic adjuvants exploit the existing antimicrobial activity inherent to the antibiotic component of the combination. This approach functions to expand antibiotic activity by identifying non-obvious synergies in non-essential gene space. Analogous to earlier studies in yeast[105,106], the biological foundation for this approach in bacteria is sound as it has been shown in *E. coli* that susceptibility to antibiotics of all classes can be substantially enhanced by the deletion of various non-essential genes[107,108], work that has been extended to other genera and species[109]. For instance, a screen of 15 different antibiotics at ¼ MIC against the KEIO collection of *E. coli* non-essential gene deletions identified 1,564 chemical–genetic interactions that enhance antibiotic activity[107]. These chemical–genetic interactions reveal rational new targets for focused drug discovery campaigns for the identification of Class Ib adjuvants. Such targets may either be specific, reflecting the species specificity of gene–gene interactions, or indicate more general suppressive or enhancing interactions[110]. With genetic and chemical–genetic interaction data in hand, Class Ib antibiotic adjuvants may be identified by computational methods that integrate complex network data to predict potential synergisms. For example, chemical sensitivity fingerprints of mutant strains have been used to predict new synergistic compounds of the folate biosynthesis inhibitors trimethoprim and sulfamethizole[111], and computational approaches to predict synergy have been applied to *Mycobacterium tuberculosis* and *S. aureus*[112].

Class Ib adjuvants can also be readily identified in forward chemical screens that are initially target agnostic. In this approach, the pathogen of interest is directly screened against libraries of non-antibiotic compounds in the presence of sub-MIC concentrations of a known antibiotic (usually ¼ MIC) to identify enhancers of cell growth inhibition. This strategy can be used to identify compounds that restore antibiotic sensitivity to otherwise antibiotic-resistant isolates[113]. Such screens have identified many interesting antibiotic–adjuvant pairs (TABLE 2). In an instructive example, a screen of off-patent drugs against *E. coli*, *P. aeruginosa* and *S. aureus* in the presence of the antibiotic minocycline identified several non-obvious adjuvant compounds[113]. One of these drugs, loperamide, a μ-opioid receptor agonist widely used as an anti-diarrhoeal therapy (known under the brand name Imodium), showed broad-spectrum ability to potentiate tetracycline antibiotics in Gram-negative bacteria in vitro and in an in vivo animal model of *Salmonella enterica* subsp. *enterica* serovar Typhimurium infection. The mode of action of loperamide as a tetracycline Class Ib adjuvant was determined to be through the disruption of the cell-membrane-associated proton motive force, which results in increased intracellular accumulation of the antibiotic and consequent enhanced inhibition of the bacterial ribosome.

Class II antibiotic adjuvants. A myriad of host defence systems can be exploited to improve the efficacy of antibiotics within infected organisms[114]. For example, immunomodulatory and cationic antimicrobial peptides have been demonstrated to be synergistic with antibiotics and can even enhance antibiotic activity in difficult-to-eradicate biofilms. Some of these peptides have intrinsic and potent antimicrobial activity[115–117], whereas others do not[118], and some even have Class Ia adjuvant activity[119]. These immunomodulatory peptides have a broad range of effects on host immune response, including suppression of inflammation to avoid overresponse to an infection that leads to sepsis and induction of host-cell-based antimicrobial activities such as enhanced phagocytosis[114,120].

Another strategy is to directly target aspects of innate immunity with small molecules. A natural product, streptazolin, capable of stimulating macrophage killing of *Streptococcus pneumoniae*, was identified in a screen of microbial natural-product extracts[121]. Streptazolin induces the production of nuclear factor-κB through the phosphatidylinositide signalling pathway, with concomitant release of anti-infective cytokines. This approach, combining antibiotics with either compounds such as immunomodulatory peptides that function in a multifaceted fashion in host immune systems, or through more targeted pathways in specific immune cells, offers an untapped target vista that may contribute to drug combination strategies to control infection. Conversely, antibiotic treatment can have effects on host metabolism that can impair antibiotic efficacy or adversely affect immune cell function[122]. For example, treatment of *E. coli*-infected mice with antibiotics such as ciprofloxacin altered the metabolism of infected tissues (for example, increased AMP levels) in a microbiome-independent fashion with further effects on immune cells such as phagocytes[122]. Finally, alteration of the growth environment such as nutrient availability for a pathogen can also radically alter sensitivity to antibiotics[123,124] such that therapeutic modulators of the host microenvironment may emerge as adjuvants in the future. Human genome-wide association studies and mouse genetic models have identified hundreds of host genetic loci that contribute to infection resistance[125]. With the recent advent of CRISPR–Cas9 genetic screening technology in human cell lines, it will be possible to systematically map host determinants that alter sensitivity to pathogen infection and antibiotic efficiacy[126]. For instance, CRISPR screens have revealed the role of a bicarbonate transporter in phagosome acidification[127] and genetic resistance mechanisms to the α-haemolysin toxin of *S. aureus*[128]. Broad application of these technologies should enable the discovery of many Class II adjuvants that mimic the effect of host resistance determinants.

Coalism

Combinations of non-antibiotic inhibitors, termed coalistic pairs[33] (FIG. 1), which target proteins corresponding to synthetic lethal genetic interaction pairs, are predicted to yield specific chemical lethality attuned to the genetic landscape of the particular pathogen of interest. Furthermore, higher-order combinations of three or more compounds in principle can mimic more complex genetic interactions, which recent evidence suggests are up to 100-fold more prevalent than pairwise interactions[129], although more complex interactions are also possible[44–46]. Higher-order compound combinations can be identified in practice by empirical tests across synergistic series and potentially by predictive methods[106] (FIG. 2b). The combinatorial strategy should overcome some limitations inherent to targeting essential gene products, including the empirical failure of the essential target strategy to identify new antibiotic drug candidates; the intrinsic vulnerability of agents that target single essential pan-species targets to selection for, and dissemination of, resistance; and the much more extensive and potentially novel target space that synthetic lethal combinations provide. Furthermore, combinations of compounds may lessen the frequency of resistance because inhibitors that target non-essential gene products lack intrinsic antibiotic activity as single agents and thus afford less opportunity for maintenance in the absence of selection for resistance in microbial populations. Finally, as network biology can often be highly species-specific[130], combination strategies offer new routes to narrow-spectrum antibiotics. Such therapies are increasingly seen as advantageous over broad-spectrum drugs that select for resistance in multiple genera and indiscriminately damage the microbiome, often with unintended consequences, such as overgrowth of *Clostridium difficile*[131] and even long-term health effects from antibiotic exposure in preterm infants[132]. A recent report demonstrates the feasibility of identifying species-specific combinations through systematic screens[133]. As point-of-care diagnostics for infections improve, such narrow-spectrum therapies will become increasingly realistic[2]. In this scenario, clinicians that know the identity of the infecting pathogen and perhaps even its drug resistance profile could turn to highly targeted drug combinations that selectively remove the offending organism with minimal damage to the host and associated microbiome.

However, targeting two non-essential but synthetically lethal gene products in coalistic antibiotic discovery does pose a theoretical issue concerning resistance: although simultaneous selection for resistance to both agents will be more difficult than with single compounds, the emergence of resistance to one agent is all that is needed to overcome the combination unless higher-order combinations of redundantly acting synergistic compounds are identified. It is instructive that natural-combination antibiotics can often exceed more than ten agents in a bioactive mixture[19,134], pointing to the use of higher-order combinations. In any case, despite success in the yeast model system for which the understanding of genetic interactions is the most advanced, there have been no notable advances to progress in antibiotic drug discovery efforts that target synthetic lethal pairs.

The knowledge of genetic network structure combined with extensive chemical–genetic interaction data sets can be used to predict compound pairs that target network vulnerabilities (FIG. 2b). Proteomic, genetic

and chemical–genetic screens can thus be mined using machine learning methods to predict non-obvious chemical combinations with enhanced activity. This strategy has been applied in the tractable *S. cerevisiae* model system for which extensive genetic and chemical–genetic data sets are available. In one recent example, many dozens of non-obvious coalistic pairs with antifungal activity were identified from input chemical–genetic interaction data sets[135,136]. In the first step, growth assays of 195 sentinel yeast gene deletion strains exposed to 4,915 small molecules revealed 1,221 chemical–genetic interactions. A chemically diverse subset of these hit compounds was used to generate a systematic matrix of 8,128 pairwise chemical combinations that experimentally identified synergistic pairs. This information was then used to train machine learning algorithms that correctly predicted dozens of unique synergistic pairs with previously unknown antifungal activity. Notably, many of these pairs exhibited species-specific effects against a panel of clinically relevant fungal pathogens. The use of machine learning approaches is in its infancy[137] and is limited by a paucity of systematic data sets for algorithm training and benchmarking[138]. Nevertheless, the rapid development of deep-learning methods, larger training data sets and accurate mathematical models of cellular processes portends future successes in the prediction of chemical synergies and resistance mechanisms[123,139–141].

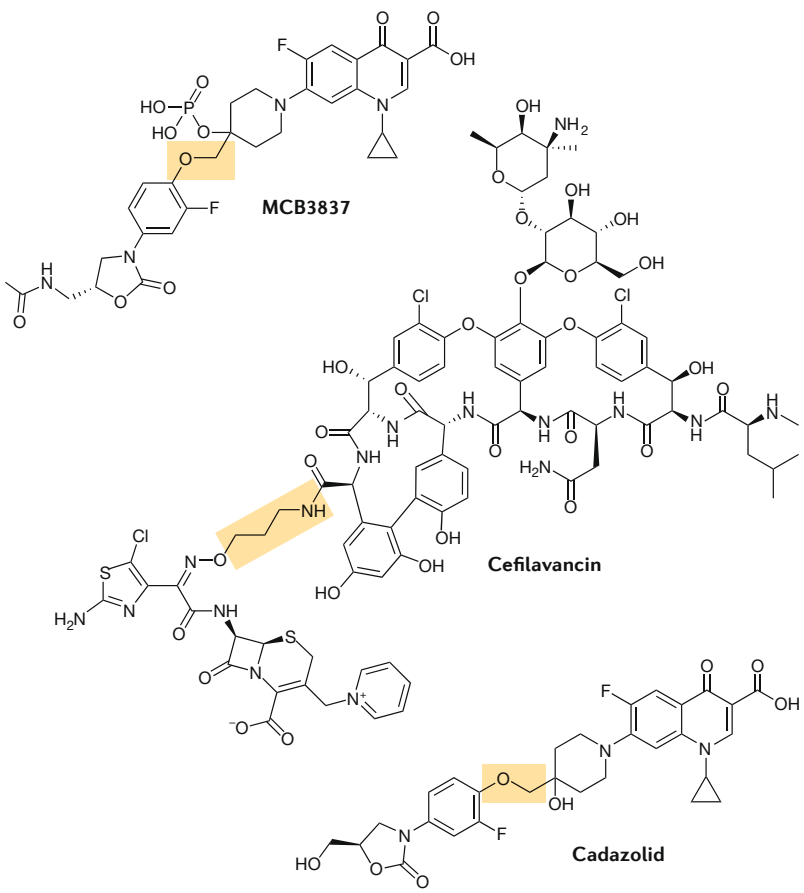


Fig. 3 | **Hybrid antibiotics.** Some of the challenges of coadministration of two synergistic agents can be overcome by synthetically linking the individual bioactive components. Shown are hybrids currently in clinical trials (PEW Trust): MCB3837 (fluoroquinolone–oxazolidinone hybrid), cadazolid (fluoroquinolone–oxazolidinone hybrid) and cefilavancin (glycopeptide–cephalosporin hybrid). Linking sections that join the individual components of each hybrid are highlighted.

Future perspectives

Congruous, syncretic and coalistic combinations afford great opportunities in the discovery and development of anti-infective medicines in the 21st century. The well-established use of congruous antibiotic combinations to achieve broad-spectrum coverage in the case where the infective organism is unknown and where the need for rapid treatment is acute remains the best argument for empirical combination of antibiotics. The disadvantage in such applications is the opportunity for unnecessary antibiotic exposure that fuels resistance in the patient and in the health-care setting. Indeed, a recent survey indicated that in India, where antibiotic controls are less rigorous than in many other countries, 188 fixed-dose congruous combinations of antibiotics are available to the consumer, only 36% of which have regulatory approval; in comparison, only 5 combinations are available in the United Kingdom and United States, all of which are approved by regulatory agencies. The unnecessary antibiotic exposure in instances of unregulated combinations, and the lack of rapid, reliable diagnostics to guide the clinician in initial therapy, are issues that can be managed with tighter regulatory controls and innovation in molecular diagnostics. However, what remain effective are synergistic congruous combinations. These combinations are proved to increase efficacy and suppress resistance, and additional efforts to identify suitable fixed-dose combinations, properly formulated, should be investigated. Such combinations will need to be powered by well-designed clinical trials, and the issue of who will sponsor such trials or pursue the development of effective drug formulations when most of the drugs in question are off-patent is an important policy question.

Syncretic combinations of antibiotics with non-antibiotic adjuvants offer a very promising area for antibiotic discovery and development. In an era when new antibiotic innovation is at a nadir, reinvigorating our existing antibiotic drug classes provides an excellent opportunity to extend the life of well-researched and clinically validated drugs. The outstanding success of the Class Ia adjuvants that block serine-β-lactamase activity is evidence that this strategy is worthy of continued exploration. Other apparent targets for Class Ia adjuvants include metallo-β-lactamases, ribosome methyltransferases that confer near-pan resistance to aminoglycosides, large ribosome subunit methyltransferases such as Erm and Cfr that confer resistance to macrolide and oxazolidinone antibiotics, respectively, and broad-spectrum efflux inhibitors, in particular of the RND class that predominates in Gram-negative pathogens. Class Ib and Class II adjuvants are not yet in clinical development, but given the success of β-lactamase inhibitors and the growing clinical need, there is excellent opportunity to pursue these as well. In particular, Class Ib adjuvants could be targeted to antibiotics that in monotherapy have failed clinical trials owing to the emergence of resistance. Much is already known

about such compounds, and these may offer highly suitable scaffolds for combination therapies. Although Class 1b adjuvants are unlikely to overcome serious deficiencies that have led to triage of such antibiotic candidates, such as unmanageable toxicity and chemical or metabolic instability, it is possible that clever deployment of Class II adjuvants may enable resurrection of abandoned antibiotics.

The development of coalistic combinations represents an unexplored frontier that is now in principle accessible through systems biology and computational approaches. We believe that higher-order combinations of three or more compounds will be needed. Although much exploratory research and preclinical development is needed, the vast landscape of genetic interactions may well be exploited in a narrow-spectrum species-specific fashion. The myriad examples in nature of combinational strategies to combat pathogens should inspire a diversity of empirical and computational approaches. In particular, the plethora of ternary genetic interactions recently described in yeast suggests that myriad higher-order combinations of compounds that mimic these interactions await discovery. These arguments resonate with the observation that effective antibiotics in nature have evolved to be impervious to resistance by virtue of activity against multiple targets[3].

The principal challenge to the successful deployment of combination strategies as new medicines lies in the complex pharmacology of antibiotic action[142,143]. Achieving the correct therapeutic levels and duration for a single antibiotic agent is already exceedingly difficult. Reaching these goals for two compounds that must be matched in terms of their pharmacokinetics and dynamics to maintain synergy considerably increases the complexity of drug development. For congruous pairs, if no historical data are available, clinical trials may need to include single agents in distinct arms of the trial. These concerns may not apply in the case of syncretic combinations, but formulation and administration may be complicated. Of course, before clinical trials, toxicology of each component and the combination must also be thoroughly investigated in case there are unexpected drug–drug interactions. The complexity rises for higher-order combinations. One solution is the synthesis of single-agent hybrids that combine, in one molecule, the bioactive domains of each component[144]. Such hybrids can suppress resistance[145] and even gain new modes of action[146]. Indeed, some are currently in clinical trials such as MCB3837 and cadazolid, both oxazolidinones–quinolone hybrids, and cefilavancin, a glycopeptide–cephalosporin heterodimer (FIG. 3). Of course, such hybrids may exhibit difficulties in cell penetration, especially in the case of Gram-negative bacteria in which porin exclusion limits the penetration of most small molecules that are >600 Da. Although not insurmountable, the synthesis of bigger compounds with more functionality is not a straightforward recipe for success.

Despite these challenges, the time is right for renewed interest and effort in developing both congruous and syncretic drug combinations to address the antibiotic resistance crisis. The development of combination therapies is more complicated than for single agents. However, the case is compelling given that monotherapy on single-target drugs leads rapidly to resistance, that finding new single-agent antibiotics has proved near fruitless for over one-quarter of a century and that all antibiotics will at some point be compromised by increasing levels of resistance. Furthermore, as alternative antimicrobial agents such as anti-virulence compounds and phage cocktails become increasingly attractive, it is very likely that these will be delivered in combination with antibiotics. Given the proven success of congruous antibiotic and antiviral combinations in the clinic today and Class Ia syncretic antibiotic–adjuvant combinations, there is excellent reason to speculate that the future of the antibiotic formulary may be dominated by combination therapeutics.

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