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Combination Approaches to Combat Multi-Drug Resistant Bacteria

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Abstract

The increasing prevalence of infections caused by multi-drug resistant bacteria is a global health problem that is exacerbated by the dearth of novel classes of antibiotics entering the clinic over the past 40 years. Herein we describe recent developments toward combination therapies for the treatment of multi-drug resistant bacterial infections. These efforts include antibiotic-antibiotic combinations, and the development of adjuvants that either directly target resistance mechanisms such as the inhibition of β-lactamase enzymes, or indirectly target resistance by interfering with bacterial signaling pathways such as two-component systems. We also discuss screening of libraries of previously approved drugs to identify non-obvious antimicrobial adjuvants.

Keywords

antibiotic resistance; adjuvant

The problem of multi drug-resistant bacteria

The emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant global public health threat. Drug resistant bacterial infections cause considerable patient mortality and morbidity, and rising antibiotic resistance is seriously threatening the vast medical advancements made possible by antibiotics over the past 70 years.[1] For example, in 2005 almost 95,000 people acquired methicillin-resistant *Staphylococcus aureus* (MRSA) infections in the United States and 19,000 people died from MRSA infections - more than die annually from HIV/AIDS, emphysema, Parkinson's disease, and homicide combined.[2] Without developing innovative approaches to combat multi-drug resistant (MDR) pathogens, many fields of medicine will be severely affected, including surgery, premature infant care, cancer chemotherapy, care of the critically ill, and transplantation medicine, all of which are feasible only with the existence of effective antibiotic therapy. Compounding the problem of rising bacterial resistance to currently approved antibiotics is a lack of investment in antibiotic discovery by the pharmaceutical industry due to the inherently low rate of return for antibiotics compared to drugs targeted at chronic diseases.[3] This situation is so dire that the World Health Organization has identified MDR bacteria as one of the top three threats to human health,[4] while the Infectious Disease Society of America has issued a call to action from the biomedical community to deal with the MDR bacterial threat.[5]

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While the development of new antibiotics is one approach for the treatment of MDR bacterial infections, the fact remains that only two new classes of antibiotics have been introduced into the clinic over the last two decades, neither of which are significantly active against Gram-negative bacteria (Box 1).[6] Furthermore, bacteria invariably develop resistance to any introduced therapy that relies solely upon a bacteriostatic/bactericidal mechanism and clinically significant resistance can appear in a period of just months to years following introduction of a new antibiotic into the clinic.[7] For example, daptomycin was introduced into the clinic in 2003, and less than a year later the emergence of resistance in patients with *Enterococcus faecium* and MRSA infections was observed.[8] As a result, alternative approaches to controlling bacterial infections are sorely needed.

Box 1

Bacteria and antibiotics

Gram-positive and Gram-negative bacteria differ in the composition of their cell envelope. The cell envelope of Gram-positive bacteria consists of an inner plasma membrane surrounded by the cell wall, a thick layer of peptidoglycan, which comprises the outermost layer of the cell. The cell wall is permeable and typically does not restrict the diffusion of antibiotics into the cell. Gram-negative bacteria meanwhile possess a much thinner peptidoglycan layer that is surrounded by a second membrane comprised of a bilayer of phospholipids and lipopolysaccharides (LPS), known as the outer membrane, which is the outermost structure of the cell. The outer membrane of Gram-negative bacteria provides an extra layer of protection for the cell as compared to Gram-positive bacteria and plays a major role in preventing the diffusion of hydrophobic molecules, including many antibiotics, into the cell. As a result, these compounds can only enter the cell through selective porins, providing an intrinsic resistance of Gram-negative bacteria to many antibiotics, despite the fact that they possess the intercellular targets of these drugs.

One such approach is the use of drug combinations to effectively combat the MDR phenotype. Such efforts include antibiotic-antibiotic combinations, and the pairing of an antibiotic with a non-antibiotic adjuvant molecule to either directly target resistance mechanisms, such as the inhibition of β-lactamase enzymes, or to indirectly target resistance by interfering with bacterial signaling pathways such as two-component systems. The screening of libraries of previously approved drugs as a means of identifying non-obvious antimicrobial adjuvants has also been explored. The purpose of this review is to provide the reader with an overview of each approach, and highlights recent advances in each area. It is not meant to be a comprehensive review of all approaches to combat antibiotic resistance, which would be beyond the scope of this document.

Combinations of two or more antibiotics

One approach to combating MDR infections is combination of two or more antimicrobial drugs during a treatment regimen. Although the possibility of drug-drug interactions is a possible pitfall to this approach, and must be taken into consideration during the drug development process, combination therapy is common and critical in many areas of medicine. For example, drug combinations are key to most cancer treatments,[9] combination therapy regimens have long been used to treat HIV infected patients,[10] and artemisinin-based combination treatments are now generally accepted as the most effective treatments for malaria.[11] Combination therapies are also important for the treatment of bacterial infections, and are used almost exclusively for the treatment of *Mycobacterium tuberculosis* infections, with combinations of up to four drugs typical.[12] The rise in occurrence of other

MDR bacteria, particularly MDR Gram-negative bacteria means that monotherapy is increasingly no longer adequate to treat these infections and is necessitating the use of combination therapies,[13] For the treatment of MDR Gram-negative infections this is often a combination of colistin, a nephrotoxic polymyxin, with another antibiotic.[14]

Antibiotic combination therapy can be divided into three categories: i) inhibition of targets in different pathways, as is the case for the combination of isoniazid, rifampicin, ethambutol and pyrazinamide for the treatment of *M. tuberculosis* infections, ii) inhibition of different targets in the same pathway, for example the combination of sulfamethoxazole and trimethoprin, (marketed as co-trimoxazole in the U.K. and various other trade names world wide), which inhibit successive steps in the folic acid biosynthetic pathway, and iii) inhibition of the same target in different ways, for example with the sterptogramins.[12b]

A recently reported example of the inhibition of different targets within the same pathway is the targeting of teichoic acid (a component of the cell wall of Gram-positive bacteria) synthesis by the natural product tunicamycin (Figure 1a).. Tunicamycin inhibits the first enzyme in the pathway of wall teichoic acid biosynthesis, the *N*-acetylglucosamine-1-phosphate transferase TarO, in *S. aureus* and displays a dramatic synergy with β-lactam antibiotics, decreasing the minimum inhibitory concentration (MIC) of oxacillin against one MRSA clinical isolate from 50 μg/mL to 0.4 μg/mL at a concentration of just 0.08 μg/mL.[15] Another TarO inhibitor was subsequently identified from a screen of a library of for the ability to potentiate the activity of the cephalosporin cefuroxime against MRSA. The antiplatelet drug ticlopidine (Ticlid) (Figure 1b) did not exhibit antibiotic activity alone, but was strongly synergistic with cefuroxime against several diverse MRSA strains, lowering MICs by up to 64-fold. This activity was shown to be dependent on the presence of the *tarO* gene, and the molecular target of ticlopidine was identified as TarO.[16] Additionally, there are several known inhibitors of early cell wall synthesis (fosfomycin, β-chloro-D-alanine, D-cycloserine, bacitracin, teicoplanin and vancomycin) at sub-inhibitory concentrations (0.25 × MIC), that exhibit significant synergy with β-lactam antibiotics and are able to bring about a reduction in methicillin resistance in a highly resistant *S. aureus* strain, effecting MIC reductions of two-fold (for β-chloro-D-alanine) to 128-fold (for fosfomycin).[17]

Antibiotic/adjuvant combinations

An alternative to the combination of two or more drugs with known-antibiotic activity for the treatment of MDR bacterial infections is to combine an antibiotic with a compound that is not, when administered alone, microbicidal but increases the activity of the antibiotic, for example by blocking the mechanism of resistance to the antibiotic. Such an approach is particularly attractive as it may also result in a decrease in the onset of resistance development. Antibiotic resistance mechanisms can be broadly divided into three categories:[7] i) Inactivation of the antibiotic, for example the enzymatic hydrolysis of β-lactam antibiotics by β-lactamases, and adenylation, phosphorylation or acetylation of aminoglycosides, ii) Removal of the antibiotic from the bacterial cell, via membrane bound efflux proteins that pump the drugs out faster than they diffuse in thereby keeping the intracellular concentration of drug lower than that required to exert the antibiotic effect. The classic example of this second mechanism is the resistance to tetracycline antibiotics in both Gram-negative and Gram-positive bacteria in which tetracycline is actively pumped out of the bacterial intracellular space, and iii) Modification of the anti-bacterial target such that the antibiotic no longer binds with high enough affinity to be effective, one such example is the production of penicillin binding protein (PBP) 2a by *S. aureus*. PBP2a has a low affinity for many β-lactam antibiotics and can therefore continue cell wall biosynthesis in the presence of levels of the antibiotic that would typically inhibit other PBPs.

The classical example of an antibiotic-adjuvant pairing is Augmentin, which combines a β-lactam antibiotic (amoxicillin) with a β–lactamase inhibitor (clavulanic acid). The addition of clavulanic acid inhibits β–lactamase activity *in vivo* and allows amoxicillin to inhibit cell wall biosynthesis. Ultimately, this combination has allowed the continued use of amoxicillin to treat infections caused by many pathogens that had developed resistance to β-lactam antibiotics.[18] Augmentin was the best-selling antibiotic in 2001, demonstrating the effectiveness of the approach of combining an antibiotic and adjuvant in clinical settings.[19] However, many β-lactamases are not inhibited by clavulanic acid and other currently available inhibitors such as tazobactam.[20] These include the carbapenem hydrolyzing oxacillinases (CHDLs) and metallo-β-lactamases (MBLs) such as the recently reported New Delhi metallo-β-lactamase (NDM-1).[21] There are several new β-lactamase inhibitors that are active against these classes of β-lactamases currently at different stages of development (Figure 1). β-Lactamase inhibitors can be broadly divided into two groups, those that contain the β-lactam core (such as clavulanic aid) and non-β-lactams. Of the first class, imidazole-substituted 6-methylidene-penem compounds such as BLI-489 (Figure 1c)[22] have demonstrated promising *in vitro* inhibition of the extended-spectrum β-lactamases (ESBLs) TEM-1 and AmpC,[4] while the tricyclic carbapenem LK-157 (Figure 1d) has also shown promising activity against various ESBLs.[23] The diazabicyclooctane NXL104 (Avibactam) (Figure 1e) is an example of a non-β-lactam β-lactamase inhibitor, and has been shown to restore cephalosporin susceptibility to a large number of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* strains.[4] Avibactam has demonstrated efficacy in animal models and in phase II clinical trials for the treatment of infections caused by Gram-negative bacteria.[24] Like other β-lactamase inhibitors, Avibactam covalently binds the enzyme but unlike β-lactam derived inhibitors is not susceptible to hydrolysis once bound to the enzyme (which would regenerate the active enzyme and destroy the activity of the released inhibitor). Deacylaction of the inhibitor/enzyme complex instead regenerates intact Avibactam, which continues to act as an inhibitor.[24] The malonate derivative ME1071 (Figure 1f) and inhibitor cocktail BAL30367[25] have shown promise in restoring the activity of β-lactam antibiotics against MBL producing bacterial strains. BAL30367 is a triple combination of the siderophore monobactam BAL19764 (Figure 1g), a bridged monobactam (Figure 1h), which inhibits class C β-lactamases, and clavulanic acid[25] and has shown good *in vitro* activity against MBL producing Enterobacteriaceae. ME107 has been shown to inhibit the MBLs IMP-1 and VIM-2, and significantly enhances the activity of the carbapenem biapenem against *Pseudomonas aeruginosa*.[4]

Compounds that inhibit efflux pumps have also been explored as adjuvants to sidestep antibiotic resistance. One early example that demonstrated the potential of this approach was the use of the known mammalian MDR pump inhibitor reserpine to suppress the emergence of resistance to ciprofloxacin in *S. aureus* and *Streptococcus pneumoniae*.[26] Several families of efflux pump inhibitors targeted at bacterial efflux pumps have since been described (Figure 1). A screening program for inhibitors of *P. aeruginosa* efflux pumps identified Phe-Arg-β-naphthylamine (PAβN), MC-207,110 (Figure 1i), which was able to inhibit all four clinically relevant pumps in this bacterium (MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXYOprM), as well as similar pumps in other MDR Gram-negative bacteria.[26–27] This compound decreased resistance to levofloxacin by eight-fold in wild-type strains of *P. aeruginosa*, while resistance was decreased by up to 64-fold in efflux pump overexpressing strains.[28] Celecoxib (Figure 1j) a nonsteroidal anti-inflammatory drug (NSAID) used in the treatment of arthritis that inhibits cyclooxygenase-2 (COX-2) has been shown to suppress drug resistance in cancers by inhibiting the MDR1 efflux pump, and has recently been demonstrated to increase the sensitivity of *S. aureus* to multiple antibiotics, including ampicillin, kannamycin, chloramphenicol and ciprofloxacin by causing an accumulation of the drugs inside the bacterial cells.[29] Virtual screening of a library of 150 celocoxib analogues using an *in silico* model for inhibition of the *S. aureus* NorA efflux pump allowed

for selection of a focused subset of six analogues for *in vitro* screening for the ability to inhibit NorA. The most active inhibitor (Figure 1k), was subsequently shown to suppress resistance to ciprofloxacin in a *norA* overexpressing strain of *S. aureus,* reducing the MIC of ciprofloxacin by 16-fold at 12.5 μg/mL.[30]

An alternative to directly inhibiting the enzyme or protein responsible for imparting resistance to the bacteria, as is the case for β-lactamase and efflux pump inhibitors, is to interfere with pathways that allow the bacteria to respond to the presence of antibiotics and activate their resistance machinery. Bacterial two-component systems (TCS) regulate the expression of genes in response to external stimuli, controlling a number of bacterial behaviors including antibiotic resistance.[31] These regulatory systems allow bacteria to sense and respond to changes in their environment and are activated by a variety of factors such as pH, nutrient level, and the presence of antibiotics. TCS consist of a histidine kinase and a response regulator. In response to an external stimulus, the histidine kinase undergoes autophosphorylation at a conserved histidine residue, and this phosphate group is then transferred to a conserved aspartate residue on the response regulator, which leads to the response.[32] The response typically involves the control of gene expression by DNA binding of the phosphorylated response regulator. Many histidine kinases can also act as phosphatases and dephosphorylate the response regulator, thus allowing precise control of gene expression in response to environmental changes.[31] TCS are ubiquitous among bacteria and possess common structural motifs not found in higher eukaryotes, potentially allowing selective, and therefore safe, targeting by small molecules. Furthermore, most TCS are not essential for bacterial growth under normal conditions, and therefore small molecule targeting of the TCS should not place selection pressure on the bacteria to mutate and become resistant to the action of the small molecule. A number of TCS are known to play a significant role in antibiotic resistance. For example, in MRSA the VraSR system is induced by exposure to several cell-wall acting antibiotics including β-lactams, glycopeptides, and bacitracin.[32] Upon induction, the VraSR system upregulates the expression of a number of genes known as the cell wall stress stimulon (CWSS), leading to increased resistance to most VraSR inducing agents. MRSA mutants that are deficient in the VraSR TCS are treatable with an oxacillin regimen *in vivo*, thus validating the potential of targeting this TCS as an antibiotic adjuvant strategy.[33] TCS are also important in the regulation of antibiotic resistance in Gram-negative bacteria. The AmgRS TCS from *P. aeruginosa* was recently identified in a screen of a transposon mutant library for increased tobramycin sensitivity. AmgRS mutations enhanced aminoglycoside action both *in vitro* and in a mouse model of infection, leading the authors to posit that small molecule targeting of AmgRS would be a promising approach to enhancing the clinical efficacy of aminoglycosides.[34] In *Acinetobacter baumannii*, the AdeRS system regulates expression of genes that encode the efflux pump AdeABC and plays a role in controlling resistance to a number of antibiotics including aminoglycosides, β-lactams, tetracyclines, erythromycin and chloramphenicol.[35] The PmrAB TCS has been implicated in resistance of *A. baumannii* to colistin, by affecting expression of genes involved in lipid A modification (resulting in a reduction in net negative charge of the outer membrane, thereby reducing susceptibility to colistin).[36] Loss of outer membrane porins (Omp), which play an important role in the penetration of antibiotics into the cell in Gram-negative bacteria, can confer resistance to cephalosporins and carbapenems, particularly in strains possessing β-lactamases. In *K. pneumoniae* the PhoBR TCS, which regulates the expression of the gene that encodes the anion selective PhoE porin, has been implicated in carbapenem resistance,[37] while the CpxAR TCS has been implicated in resistance to several β-lactam antibiotics, in addition to chlormaphenicol, and has been shown to control expression of the efflux transporter genes *acrB*, *acrD* and *eefB*.[3838] Given this ubiquitous involvement in antibiotic resistance, the bacterial TCS represents a potentially powerful, and thus far unexploited, antibiotic adjuvant target for small molecule development.

Despite this adjuvant potential, many of the early inhibitors of the TCS were investigated as potential antimicrobial agents.[39] One such example is the phenethylguanidine containing histidine kinase inhibitor RWJ-49815 (Figure 2a). This compound is a potent bactericidal Gram-positive antibiotic, and exhibited MICs in the 1–2 μg/mL range against several strains drug resistant bacteria including MRSA, vancomycin-resistant *E. faecium*, and penicillin-resistant *S. pneumoniae*. RWJ-49815 has been demonstrated to inhibit the autophosphorylation of kinase A of the KinA:Spo0F two-component signal transduction system *in vitro* and displayed reduced resistance emergence in a laboratory passage experiment in comparison to conventional antibiotics.[39b] RWJ-49815 and analogues demonstrate the feasibility of targeting TCS with small molecules, and while no compounds that have been directly shown to directly inhibit TCS signaling involved in antibiotic resistance have yet been reported as antibiotic adjuvants, a number of adjuvant compounds have been reported to be dependent upon TCS systems.

A number of 2-aminoimidazole containing compounds derived from the marine natural products oroidin and bromoageliferin have been reported to suppress resistance of both Gram positive[40] and Gram-negative[40a, 41] bacteria to β-lactam antibiotics (Figure 2b–c). The compound in Figure 2b suppressed resistance of a New Delhi metallo-β-lactamase (NDM-1) producing strain of *K. pneumoniae* to carbapenem antibiotics, lowering MICs by 16-fold. A related series of compounds (shown in Figure 3) suppressed resistance of MRSA to oxacillin, and it has been recently reported that the activity of the lead compound from this series, Figure 2c, which suppressed resistance in a number of MRSA clinical isolates by up to 512-fold, was dependent on the VraSR TCS.[40e]

Screening of previously approved drugs

The redeployment of drugs that have previously been approved for other indications is an attractive approach to the discovery of antibiotic adjuvants because these drugs have known toxicology and pharmacology profiles. It is estimated that by taking this approach, the overall cost of bringing a drug to market can be reduced by almost 40% as a result of elimination of much of the toxicological and pharmacokinetic assessment required for drug approval.[42] Systematic screening of approved ‘non-antibiotic’ compounds for anti-bacterial activity identified compounds from a number of drug classes including antihistamines, tranquilizers, antihypertensives, antispasmodics, and anti-inflammatory drugs, that displayed activity against a broad-spectrum of bacteria.[43]

Application of this approach to the search for antibiotic adjuvants, a screen of 1,057 previously approved drugs in combination with for enhancement of activity of the semi-synthetic tetracycline minocycline against *S. aureus, E. coli* and *P. aeruginosa* revealed 69 non-antibiotic compounds that potentiate the antibiotic activity of minocycline. Against *S. aureus*, including several MRSA strains, disulfiram (Antabuse) shown in Figure 3a, exhibited a strong synergistic effect, while benserazide (Figure 3b), and loperamide (Immodium, Figure 3c), increased the susceptibility of several MDR strains of *P. aeruginosa* to minocycline (Figure 3). The combination of loperamide and minocycline was also synergistic against *E. coli* and strains of several other Gram-negative pathogens including *A. baumannii, K. pneumoniae* and *Salmonella enterica*. Membrane permeability of *E. coli* and *P. aeruginosa* was increased in the presence of loperamide, suggesting a possible mechanism of action for the adjuvant activity of this compound. *In vivo* activity of the loperamide-minocycline combination was demonstrated in a mouse model of infectious colitis caused by *S. enterica* Typhimurium.[44]

The NSAID diclofenac sodium (Figure 3d), marketed in the topical anti-inflammatory gel Voltaren, possesses antibacterial activity against strains of *S. aureus, Listeria*

monocytogenes, *E. coli*, and Mycobacterium spp., including drug-resistant strains. Diclofenac enhanced activity of streptomycin against *S. aureus, E. coli* and Mycobacterium spp., and gentamicin against *L. monocytogenes*. Diclofenac also displayed synergy with streptomycin in mouse models of both *S. typhimurium* and *M. tuberculosis* infection.[43] As mentioned earlier another NSAID, celecoxib (Figure 1j), has been shown to increase the sensitivity of *S. aureus* to multiple antibiotics, while the antiplatelet drug ticlopidine (Figure 1b) was identified as an inhibitor of wall teichoic acid synthesis in MRSA from a screen of 2080 previously approved drugs.[16]

Several phenothiazine derived drugs (Figure 3) have been shown to possess anti-bacterial activity in addition to exhibiting synergy with clinically used antibiotics against a broad spectrum of bacteria, including, Gram-positive, Gram-negative and mycobacterium.[45] The antipsychotic phenothiazine thioridazine (Figure 3e) has been demonstrated to increase the susceptibility of MRSA to oxacillin[46] and dicloxacillin[47] in a number of clinical isolates.[47] It was shown that the oxacillin-induced transcription of *mecA* and expression of PBP2a[46a] is reduced in the presence of thioridazine, as is transcription of several genes belonging to the VraSR regulon.[46b] Thioridazine has also long been known to exhibit antibiotic effects against *M. tuberculosis*,[48] including multi-drug resistant strains, though not at clinically relevant concentrations.[49] Subsequent investigation of thioridazine for the ability to enhance susceptibility of *M. tuberculosis* to clinically used antibiotics revealed that thioridazine enhanced the activity of rifampicin and streptomycin against several *M. tuberculosis* clinical isolates.[50] Demonstrating the potential of this approach as a therapeutic option, evaluation of thioridazine in a mouse model of tuberculosis revealed that thioridazine displayed both significant reduction in CFU when administered alone, and synergism with a treatment regimen consisting of rifampicin, isoniazid and pyrazinamide.[51] Another phenothiazine, the anti-histamine promethazine (Figure 3f), which does not possess antibacterial activity when administered alone, has been shown to exhibit significant synergistic activity with penicillin G against *E. coli*.[52]

As mentioned earlier, the lack of novel antibiotics that are effective against Gram-negative bacteria is a significant problem. This has led clinicians to the view that for Gram-positive infections, we need better drugs, but for Gram-negative infections we need any drugs. Gram-negative bacteria are intrinsically insensitive to a number of antibiotic classes despite possessing the targets upon which these antibiotics act; examples include the macrolides and aminocoumarins. This is predominantly a result of the low permeability of the outer membrane of Gram-negative bacteria. One novel approach to circumvent this lack of antibiotics that are active against Gram-negative bacteria is the identification of adjuvant molecules that potentiate the effects of Gram-positive active antibiotics against Gram-negative bacteria. A recent reported screen by the Wright group of 30,000 compounds from the Canadian Compounds Collection identified four compounds (Figure 3g–j) that displayed synergism with novobiocin against *E. coli*. Mechanistic studies revealed all four compounds affected cell shape and membrane permeability, providing a physical basis for this synergy.[53]

Conclusions and Outlook

With the continuing rise in occurrence of drug resistant strains of bacteria, new approaches to combating infections caused by these bacteria are desperately needed, particularly for Gram-negative bacteria. The one drug-one target model has limited viability and combination therapy is the norm in the treatment of many cancers, viral infections such as HIV and tuberculosis treatment. The use of combination therapy, or other drug cocktails such as antibiotic/adjuvant combinations for the treatment of other MDR bacterial infections is an attractive alternative to the development of new antibiotics, which has been

demonstrated to almost invariably lead to the emergence of resistance following a short time in the clinic. The use of antibiotic-adjuvant combinations presents several advantages over the development of new antibiotics, namely the decreased likelihood of resistance development. Several approaches have been discussed here, including recent developments towards traditional adjuvants such as β-lactamase inhibitors and efflux pump inhibitors, new approaches such as interference of the bacterial response to the presence of antibiotics by the targeting of bacterial signaling pathways, and the use of high throughput screening of previously approved drugs to identify compounds with unanticipated adjuvant activity. There are issues with the use of any drug combinations, most notably drug-drug interactions and optimized drug ratios and dosing regimens to match the ADME (adsorption, distribution, metabolism, and excretion) properties of each compound. However, the lack of novel antibiotic classes being developed, the significant problem of resistance acquisition to therapeutic approaches that rely solely upon a single bacteriostatic/bactericidal mechanism, and the clinical success of antibiotic/adjuvant combinations such as Augmentin makes the adjuvant approach an extremely attractive avenue to the development of novel therapeutics to treat multidrug resistant bacterial infections.

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Highlights

- The problem of drug-resistant bacterial infections continues to worsen
- Bacteria will inevitably acquire resistance against new antibiotics
- Antibiotic-adjuvant combinations are an attractive approach to treat infections

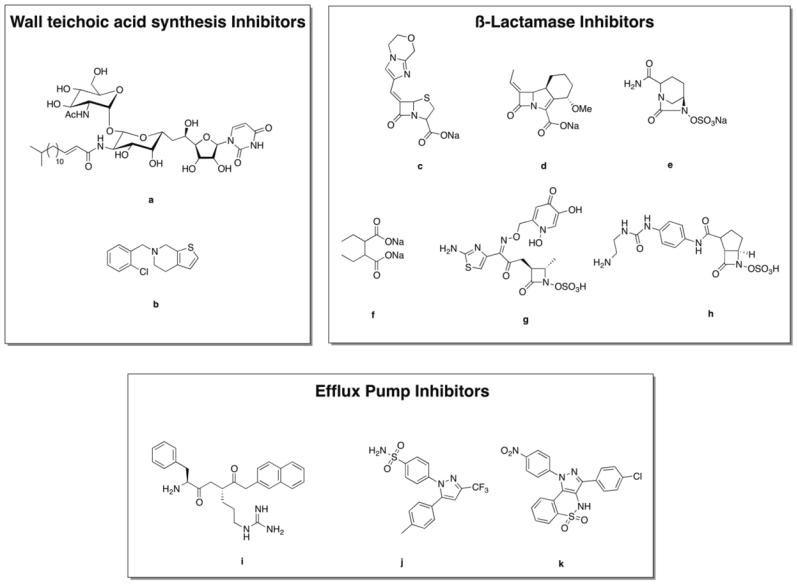

Figure 1.

Combination therapy and traditional adjuvant targets. Wall teichoic acid sysnthesis inhibitors a) tunicamycin and b) ticlopidine, c–h) β-lactamase inhibitors and i–k) efflux pump inhibitors.

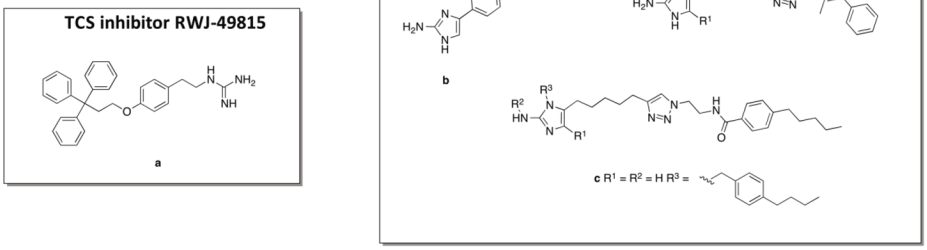

Figure 2.
Targeting two-component systems. a) The TCS inhibitor RWJ-49815 and b–c) 2-aminoimidazole compounds that suppress antibiotic resistance

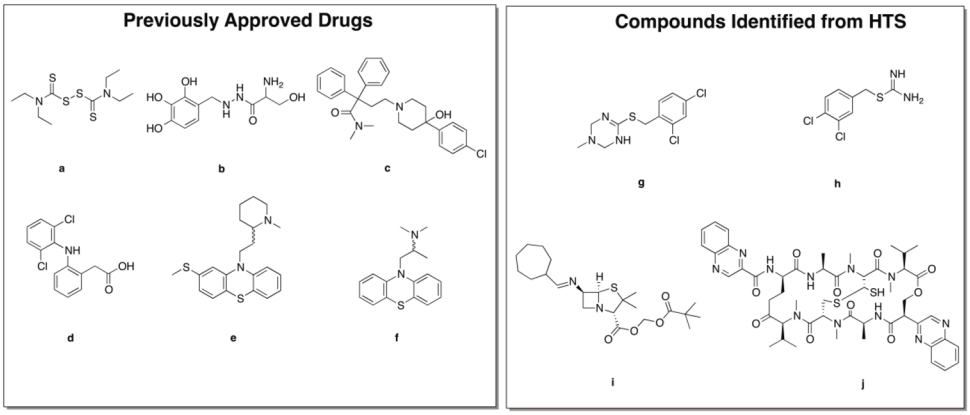

Figure 3.
a–f) Approved drugs and g–j) compounds identified from high throughput screens that display adjuvant activity