Resisting resistance: new chemical strategies for battling superbugs

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As microbes become increasingly resistant to antibiotics, and in many cases to several drugs simultaneously, the search is on to find new therapies. One method to combat resistance is to use inhibitors of resistance mechanisms to potentiate existing antibiotics. Recent efforts are encouraging and highlight the importance of research at the chemistry–microbiology interface in developing new approaches to tackle resistance.

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Antibiotic resistance is a growing problem in the treatment of infectious diseases caused by bacteria, fungi, parasites and viruses. In particular, bacterial resistance to antibiotics has emerged over the past decades as a major health problem [1,2]. This is especially true in hospitals and chronic care facilities, which provide strong selective pressure for the emergence of resistance because of the large quantities and the variety of antibiotics used in these environments. All widely used antibiotics are now subject to bacterial resistance and even some of the newer agents such as the streptogramins and the new generation fluoroquinolones are finding a significant established resistance levels as a result of similar compounds being used in medicine and agriculture during the past several years.

The challenge of antibiotic resistance has generally been met in two ways: through the discovery of completely novel antibiotics and by the use of derivatives of known antibiotics — frequently prepared by semisynthetic methods — that are not affected by existing resistance mechanisms. Although completely novel classes of antibiotics were discovered and frequently implemented rapidly into clinical practice throughout the 1940s and 1950s, a truly new antibiotic class has not emerged for many years, perhaps since the discovery of the fluroquinolones in the early 1970s, which themselves were derived from nalidixic acid discovered in the early 1960s. This trend may now be changing with the advent of several new antibacterial compounds, including the oxazolidinones such as linezolid [3] and the lipopeptide daptomycin [4]. In contrast, the variation or modification of known antibiotics has been the major source of new medicinal agents for decades. New β-lactams (e.g. methicillin and oxacillin), aminoglycosides (e.g. tobramycin and amikacin) and other antibiotics, very often prepared by semisynthetic methods, have been the mainstay of antibiotic drug discovery.

An alternative to these two pathways towards new therapies as a response to antimicrobial resistance is the development of inhibitors of resistance mechanisms [5]. In this approach, the antibiotic is co-administered with an inhibitor that neutralizes the resistance mechanism and consequently the antibiotic is still useful, even in resistant organisms. This approach has the advantage of extending the utility of antibiotics of known pharmacology, toxicology, treatments schedules and so on, long after resistance emerges. Given that there have been only a few new antibiotics brought to clinical trials over the past decade, despite the general agreement that such compounds are greatly needed, this approach should find increasing favor.

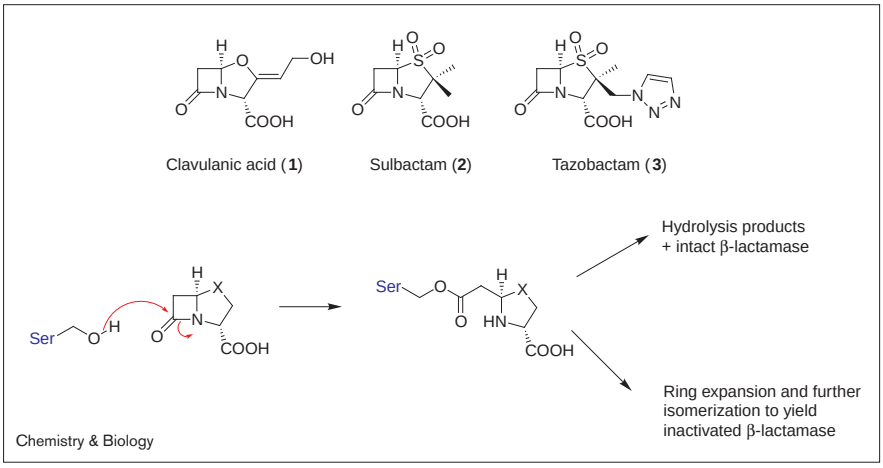
β-Lactams

The primary route of resistance to the β-lactam antibiotics is through the production of hydrolytic enzymes termed β-lactamases [6]. There is well-established clinical precedent for the use of resistance inhibitors as potentiators of antibiotic action in the β-lactam field [7,8]. Clavulanic acid (**1**), sulbactam (**2**) and tazobactam (**3**) are β-lactam compounds with only weak antimicrobial activity (Figure 1). They are, however, inhibitors of nonmetallo β-lactamases (principally of class A, Bush group 2) and are clinically administered in conjunction with a β-lactam antibiotic such as amoxicillin, ampicillin or piperacillin. These inhibitory compounds act as covalent slow-dissociating inhibitors of many serine β-lactamases through acylation of the active-site serine (Figure 1). The β-lactam/inhibitor combinations have been in clinical use for more than 15 years and find use in the treatment of infections caused by a variety of both gram-negative (e.g. *Escherichia coli* and *Haemophilus influenzae*) and gram-positive (e.g. *Staphylococcus aureus* and *Bacteroides sp.*) bacteria. Not surprisingly, the β-lactam/inhibitor combinations also are susceptible to resistance and β-lactamases that can readily hydrolyze the inhibitors have emerged [9]. Consequently, efforts to circumvent this emerging resistance through the chemical modification of the inhibitors (e.g. [10]) or the development of novel inhibitors (e.g. [11]) are under way.

Aminoglycosides

Resistance to the aminoglycoside antibiotics occurs primarily through regiospecific chemical modification catalyzed by O-phosphoryltransferases, N-acetyltransferases

Figure 1



Structure and general mechanism of action of medically important β-lactamase inhibitors.

and O-adenyltransferases [12]. The broad dissemination of these resistance mechanisms in pathogenic bacteria has limited the use of aminoglycoside antibiotics such as kanamycin, which is sensitive to all three mechanisms. Traditionally, new compounds such as tobramycin, a kanamycin analogue that lacks a key site of chemical modification (3′-hydroxyl) yet retains antimicrobial activity, have been introduced in response to the growing resistance problem.

The alternative approach of combining aminoglycosides with inhibitors of specific aminoglycoside resistance enzymes has been demonstrated to be effective in experiments reported almost 20 years ago. The natural product 7-hydroxytropolone (**4**; Figure 2) and derivatives were shown to be micromolar inhibitors of ANT(2″), an aminoglycoside adenyltransferase widely found in gram-negative pathogens [13]. Furthermore, compound **4** reversed tobramycin and dibekacin resistance in *E. coli* harboring the *ant(2″)* gene, establishing the usefulness of this strategy.

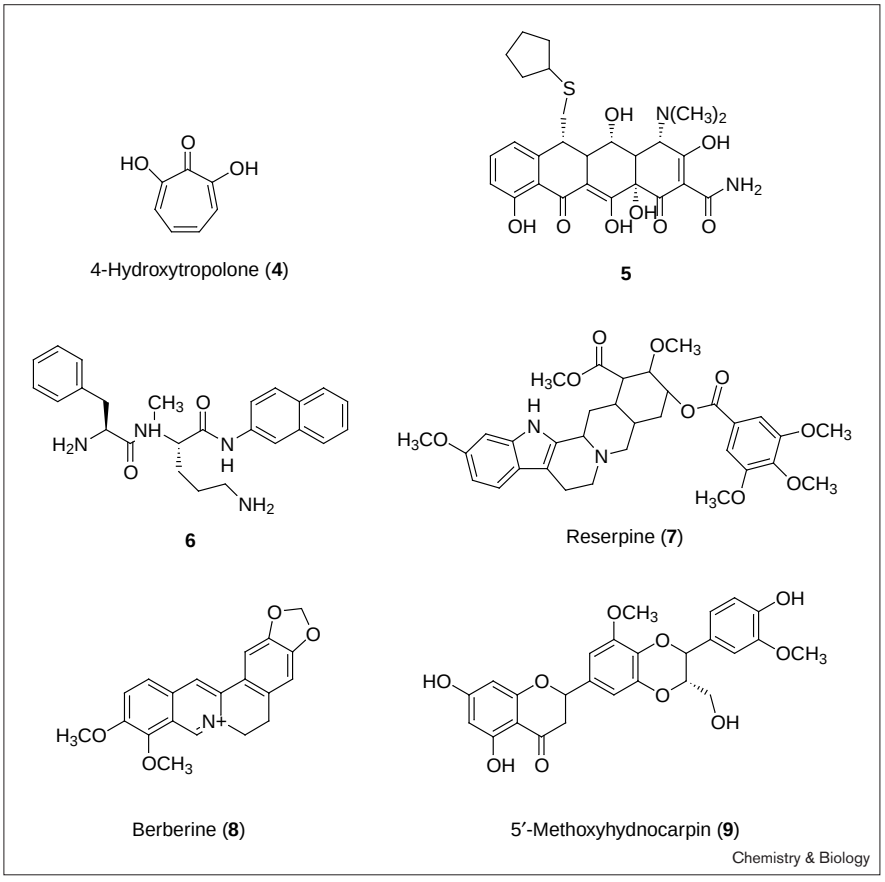
Mobashery and colleagues at Wayne State University [14] have prepared several novel aminoglycosides with the potential to either inhibit or evade the action of resistance enzymes. The synthesis of 2′-nitro derivatives of aminoglycosides was the first example of mechanism-based inactivation of an aminoglycoside-resistance enzyme. These compounds are substrates for the widespread APH(3′) enzymes that phosphorylate a hydroxyl group at position 3′, which is vicinal to the 2′NO_2. Phosphorylation of 3′OH generates compounds that can readily undergo nonenzymatic elimination to generate the nitroalkene, which presumably can undergo Michael addition by active-site nucleophiles (Figure 3). Although these compounds were not effective in reversing aminoglycoside resistance in bacteria, the technology could be developed for use in other contexts, as it should be applicable to a variety of group transferases.

Very recently, the same group has reported the synthesis of a 3′-oxo-aminoglycoside as a means of evading the action of APH(3′) kinases [15]. In solution, the 3′-keto derivative is expected to be hydrated and therefore is a potential substrate for APH(3′). The resulting 3′-phospho-derivative is, however, unstable as a result of the ketone-diol equilibrium and the leaving group nature of the phosphate. The antibiotic is therefore readily regenerated nonenzymatically (Figure 4). The compound does not seek to inhibit resistance enzymes as a means to potentiate antibiotics; instead, this aminoglycoside is simply not affected by the presence of resistance enzymes through a bit of clever chemistry. The 3-keto derivative of kanamycin was not as effective an antibiotic as the parent compound, as assessed by its MIC (the concentration of antibiotic required to achieve complete inhibition of growth), which was 250 μg/ml versus 8 μg/ml for kanamycin. The ketone did, however, lower the MIC 4–8-fold in the presence of the resistance enzyme APH(3′)-Ia. Unlike the next-generation antibiotics that seek to evade resistance by being poor substrates for existing resistance enzymes, the use of self-regenerating antibiotics such as 3′-keto-aminoglycosides could reduce the selective pressure that fuels the evolution of resistance mechanisms.

Finally, as representative three-dimensional structures emerge for aminoglycoside-resistance enzymes, new opportunities are arising to exploit these in inhibitor design. For example, the structural and functional homology between APH(3′)-IIIa and Ser/Thr/Tyr kinases [16,17] is paralleled by sensitivity to several inhibitors of protein kinases [18]. Ongoing synthetic efforts to develop libraries of protein kinase inhibitors in the pharmaceutical

Figure 2

Structures of compunds referred to in the text that inhibit antibiotic resistance mechanisms or have antimicrobial activity.



and biotechnology industries could therefore simultaneously generate compounds with the capacity to reverse antibiotic resistance, if screened appropriately.

Macrolides

Resistance to the macrolide antibiotics such as erythromycin and azithromycin occurs primarily as a result of specific base methylation of the bacterial 23S ribosomal RNA catalyzed by the Erm family of methyltransferases [19]. Furthermore, this site-specific methylation at the peptidyl transfer site on the ribosome also confers resistance to the lincosamide antibiotics such as clindamycin and the B class streptogramins antibiotics such as quinupristin, a component of the drug Synercid. Efforts to

Figure 3

Proposed inactivation of aminoglycoside 3′-kinases by 2′-nitro aminoglycoside derivatives.

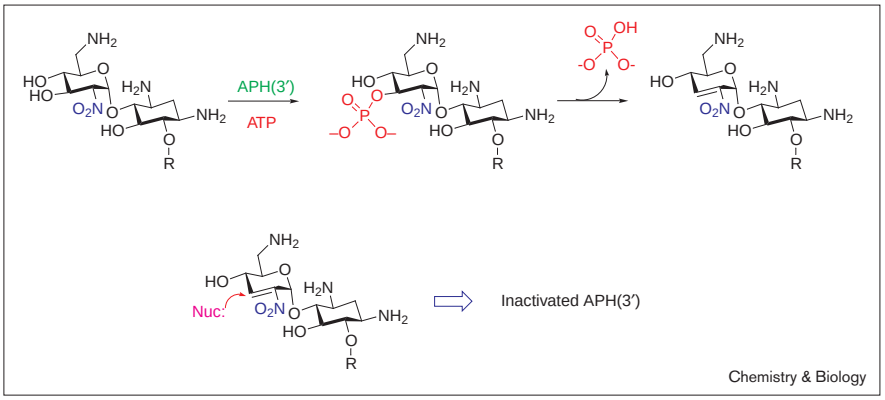
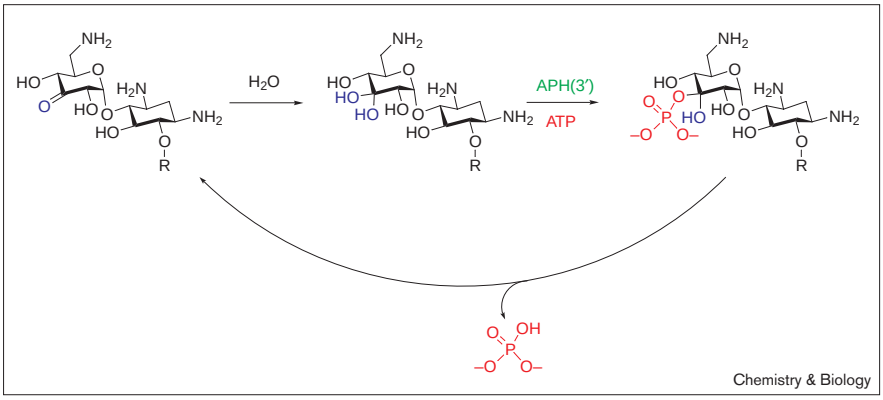


Figure 4



Self regeneration of 3′-ketoaminoglycosides following phosphorylation by aminoglycoside 3′-kinases.

discover drug-like molecules capable of inhibiting the Erm methytransferases have been reported recently. A screen of 160,000 compounds yielded nine novel chemicals that inhibited ErmC methyltransferase from *S. aureus*, five with IC_{50} values of <5 μM [20]. Some of these compounds in combination with the macrolide antibiotic azithromycin demonstrated synergistic growth inhibition against several bacteria, demonstrating that the approach of inhibitor/antibiotic combinations is a feasible route to combat Erm-based resistance.

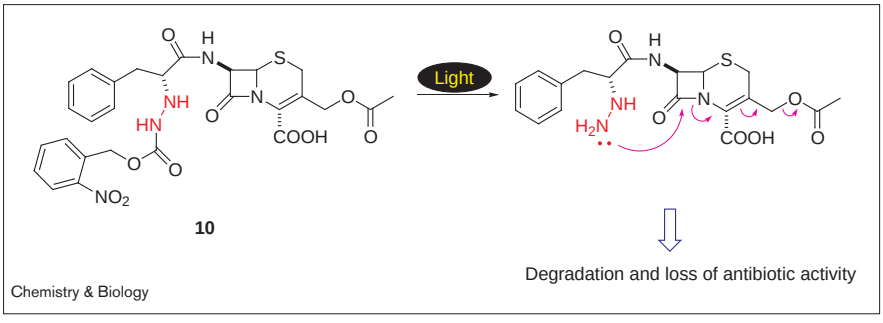
Recently, the group of Fesik at Abbott laboratories [21,22] used their SAR by NMR (structure-based activity relationship by nuclear magnetic resonance) method, in which mixtures of small molecules are probed for interaction with a target enzyme by monitoring changes in the $^{15}N/^{1}H$ chemical shifts to identify several leads for Erm inhibitors [23]. The study therefore yielded several classes of new compounds that could be used to potentiate the antimicrobial activities of the macrolide lincosamide and streptogramin B classes of drugs in the presence of Erm methyltransferases.

Efflux mechanisms

Efflux plays an important role in resistance not only to antibacterial agents such as the fluoroquinolones and tetracyclines, but also to antifungal, antimalarial and anticancer drugs as well [24]. Membrane-bound pumps of both the multidrug resistance ABC transporter and proton-dependent major facilitator families mediate resistance by efflux. These pumps can have narrow or broad compound specificities and are widespread in both gram-negative and gram-positive bacteria. Inhibition of efflux pumps as a mechanism of potentiating antibiotic activity has been an area of vigorous research.

Tetracycline resistance occurs either through active efflux or by protection of the ribosomal target [25]. Analogues of tetracycline, including 13-cyclopentylthio-5-hydroxy-6-dexoytetracycline (**5**), have been shown to inhibit the efflux protein TetB in everted membrane vesicles [26]. Recently, this compound has been demonstrated to potentiate the antibiotic activity of doxycycline against *E. coli* bearing either the closely related TetA or TetB efflux systems, but not against the gram-positive organisms *S. aureus* or *Enterococcus hirae* expressing the more distantly related TetK or TetL efflux pumps [27]. These results are encouraging but also reveal the challenges that multiple resistance determinants present to efforts to provide broad-spectrum strategies for reversal of resistance.

Figure 5



Light inactivation of a β-lactam antibiotic.

In addition to the problem of multiple resistance genes found in different organisms, certain bacteria are known to express several different efflux systems [28,29]. For example, four such systems have been identified in the opportunistic pathogen *Pseudomonas aeruginosa*: MexAB-OprM [30], MexCD-OprJ [31], MexEF-OprN [32] and MexXY [33]. Compounds that simultaneously inhibit all three mechanisms have been recently identified using compound library screening followed by lead optimization by researchers at Microcide Pharmaceuticals and Daichi Pharmaceutical Company [5]. The dipeptide **6** (Figure 2) was shown to potentiate the antipseudomonal activity of the fluoroquinonlone levofloxacin *in vitro*, and it was also effective in a mouse model for *P. aeruginosa* infection.

The major multidrug-resistance pump in *S. aureus* responsible for fluoroquinolone resistance is NorA, a chromosomally encoded multidrug-resistance pump that confers resistance upon overexpression [34,35]. The natural product reserpine (**7**; Figure 2) has been known for a number of years to inhibit the action of the NorA [34] and to potentiate the action of the fluoroquinolone antibiotic norfloxacin [36]. An interesting and important finding in these studies is that not only did resperpine reduce the MIC for norfloxaxin, but addition of the NorA inhibitor to the growth medium also decreased the rate of emergence of high-level resistance [36]. If this observation is found to be generally applicable, then this provides yet more impetus for the discovery and implementation of inhibitors of resistance.

The neurotoxic properties of reserpine prompted a search for small-molecule inhibitors of NorA using a commercial compound library [37]. A screen of 9600 compounds resulted in five candidate inhibitors of NorA, all of which could potentiate the activity of the fluoroquinolone antibiotic ciprofloxacin against *S. aureus*. Furthermore, these compounds also retained the capacity to diminish the rate of emergence of fluoroquinolone resistance, mirroring the properties of reserpine.

Although the mechanism-based and random screening approaches to the discovery of antibiotic potentiating molecules are proving very successful, a creative approach to the problem was recently reported by the Lewis lab at Tufts University [38]. These researchers previously observed that the antistaphylococcal properties of the plant alkaloid berberine (**8**; Figure 2) were significantly increased in the absence of NorA [35]. They then reasoned that, as alkaloids such as berberine are substrates for bacterial efflux pumps, perhaps plants may have co-evolved efflux inhibitors that could preserve the antimicrobial activity of the compounds. A screen of leaf extracts of the berberine producer *Berberis fremontii* resulted in the isolation of 5′-methoxyhydnocarpin (**9**; Figure 2), a potent NorA inhibitor that potentiated the antimicrobial activity of berberine (16-fold decrease in MIC) and norfloxacin (fourfold decrease in MIC) against *S. aureus* [38]. These results reveal that plants have the potential to use compound synergy to increase the potency of secondary metabolites, and alert us to the utility of natural product screens in the search for potentiators of antimicrobial activity.

Antibiotic volatility

One of the driving forces of the maintenance of resistance is the veritable pollution of hospital and agricultural environments with antimicrobial compounds, which are generally chemically stable, long-lasting molecules. This provides constant selective pressure for the maintenance of resistance genes by bacterial populations exposed to these compounds. Recently, an innovative approach to decreasing resistance has emerged from the lab of Shahriar Mobashery. This group has synthesized and characterized a photolabile β-lactam antibiotic (**10**; Figure 2). The synthesis of a cephalosporanic acid derivatized with a hydrazine functionality at position C7b that is masked by a light sensitive o-nitrobenzyloxycarbonyl group provides a built-in light-activated 'autodestruct timer' for the inactivation of the antibiotic. Loss of the o-nitrobenzyloxycarbonyl upon exposure to light reveals the hydrazine moiety, which can now participate in an intermolecular ring expansion with the β-lactam ring, precipitating degradation of the antibiotic into non-antimicrobial byproducts (Figure 5). This approach provides an excellent example of the creative application of novel chemistry to reduce the selective pressure generated by the tons of antimicrobials released into the environment every year.

Future prospects

The examples cited in this minireview show that new strategies to combat antibiotic resistance are emerging through innovative approaches to develop new antibiotics or to discover potentiators of antimicrobial action.

There is no definitive cure for antibiotic resistance. Faced with the relentless power of natural selection, only a continuing strategy of vigilance, creativity and preparedness will ensure that the antibiotic era continues well into this century.

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