Expert Opinion

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Drug delivery approaches to overcome bacterial resistance to β-lactam antibiotics

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Background: Since its landmark discovery in 1928, penicillin has had a profound impact on the quality of human life. The ability to treat and cure deadly infections and bacterial diseases has forever changed our medical profession and way of life, providing unprecedented relief from pain, suffering, and death due to microbial infection. Penicillin and its many derivatives have dominated the field of antibiotics research and development, while demonstrating unprecedented success as a therapeutic used around the world. The β-lactams, as a family of more than six structural variants all having the 2-azetidinone ring, have worked extremely well against a wide variety of disease-causing pathogens, while exerting little if any toxicity towards mammalian cells. Penicillin has truly been a wonder drug. However, over the last 60 years, drug resistance to the penicillins has steadily been increasing in frequency and severity, to the point where today there are grave concerns that the β-lactams will soon no longer be able to stop deadly bacterial infections. *Objective*: The aim of this discussion is to present what has been investigated as a means to enhance the performance of β-lactam antibiotics against drug-resistant bacteria, and what is currently being explored or is likely to prove useful in the future. *Methods*: This review provides a descriptive overview of the various published ways to enhance the clinical effectiveness of β-lactam antibiotics, beginning with the early and ongoing search for more powerful β-lactam derivatives, penicillinase-stable variants, β-lactam prodrugs, intracellular delivery approaches, nanocarrier-based strategies, and new β-lactams with an alternative mechanism of action. *Conclusion*: Of the progress made so far to develop approaches to overcome bacterial resistance to β-lactams, the use of drug carriers such as liposomes and nanoparticles seems to hold significant promise, as do structural variants that operate through different biological modes of action.

Keywords: antibiotic resistance, β-lactamases, β-lactams, drug delivery, nanoparticles, penicillin

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1. Introduction

β-lactams are a class of antibiotics having as their most distinctive feature a reactive four-membered azetidin-2-one ring, which is the source of biological activity [1]. The family includes the bicyclic penicillins, cephalosporins and carbapenems, as well as the monocyclic nocardicins and monobactams (**Figure 1**). Obtainable in large scale through fermentation and semisynthetic modification, these compounds are relatively inexpensive to produce, are amenable to chemical derivatization, are relatively stable in storage, can be delivered orally, and are highly effective chemotherapeutic agents for treating a wide assortment of

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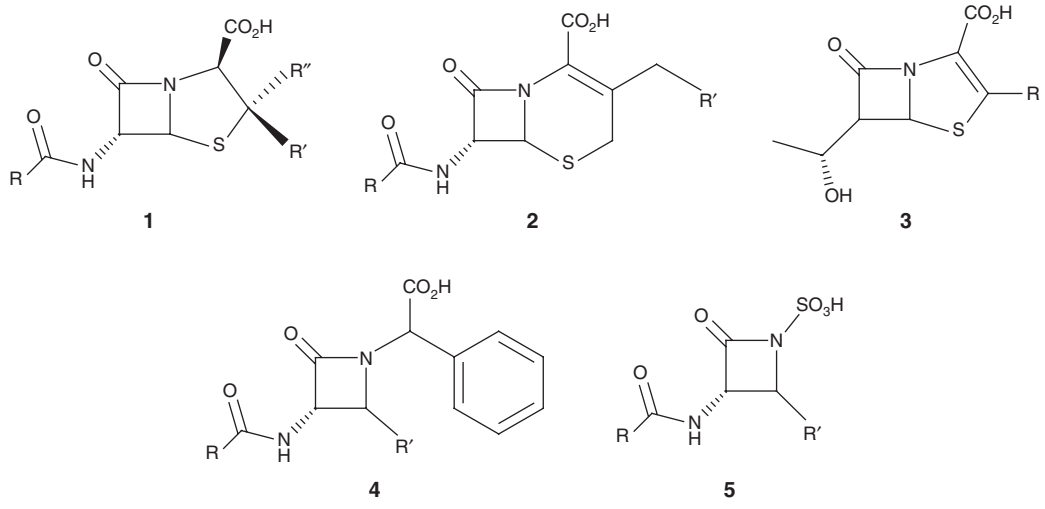


Figure 1. The family of β-lactam antibiotics: penicillins (1), cephalosporins (2), carbapenems (3), nocardicins (4) and monobactams (5).

microbial infections [2-3]. The β-lactam antibiotics are bactericidal agents that inhibit bacterial cell wall synthesis. Both Gram-positive and Gram-negative bacteria are surrounded by a cell wall, composed mainly of a peptidoglycan: a strong net-like polymer responsible for maintaining the shape and size of the bacterial cell and for resisting the high intracellular osmotic pressure. The cell wall also serves as an effective barrier to separate the contents inside the cell from the extracellular environment. The glycan component of this rigid cell wall structure consists of alternating units of *N*-acetylmuramic acid and *N*-acetylglucosamine, the former having short peptide stems attached to it on the nitrogen center. In cell wall biosynthesis, the peptide fragments in adjacent glycan strands are enzymatically crosslinked together, producing the characteristic web-like structure of the peptidoglycan. These final steps of peptidoglycan biosynthesis occur extracellularly through biochemical processes that are mediated by enzymes associated with the cellular membrane. Most commonly referred to penicillin-binding proteins (PBPs), their main function is to crosslink peptide chains between adjoining glycan strands. Penicillin is morphologically similar to the D-alanyl-D-alanine terminus of the pentapeptide attached to *N*-acetylmuramic acid, and fits neatly inside the active site of the PBP. It is here that penicillin irreversibly binds to the protein by acylating the catalytic serine residue. This event disrupts cell wall synthesis by blocking trans-peptidation of the nascent peptidoglycan, thus causing the bacterial cells to become more permeable to water and eventually lyse open [4-7].

β-lactam antibiotics have been in clinical use for > 60 years and their extensive availability in hospitals and the community has created major environmental pressures for bacteria to evolve towards resistance. Penicillin resistance is nothing more that the microbe's evolutionary response to fight for survival as a means to avoid being killed off by the therapeutic agent [8]. This acquired resistance to antibiotics is common in pathogenic bacteria by way of four known mechanisms of resistance. The primary resistance comes from a collection of defense enzymes known as β-lactamases, which are capable of hydrolyzing the β-lactam ring to convert the antibiotic to an inactive metabolite [9]. Bacterial resistance against penicillins, cephalosporins, monobactams and carbapenems is most often mediated by β-lactamases, which have emerged and evolved rapidly in both Gram-positive and Gram-negative bacteria [10]. The second mechanism of resistance is genetic alteration of the actual antibiotic target site, the PBPs [11]. This is now a major cause of resistance in several pathogens including the highly problematic Gram-positive *Staphylococcal* and *Streptococcal* species. There were 8987 observed cases of invasive methicillin-resistant *Staphylococcal aureus* (MRSA) reported in USA from July 2004 through December 2005 [12]. The third resistance mechanism is an altered permeability of the cellular membrane causing a more highly restricted access of the antibiotic to the target protein [13]. The final resistance factor is associated with forced efflux of the antibiotic from the cytosol, a feat performed by the MexA, B-OprM antibiotic efflux pump, which is a major cause of resistance in *Pseudomonas* and other pathogenic Gram-negative bacteria [2,14].

There are likewise four known classes of β-lactamases, the primary cause of acquired resistance to β-lactam antibiotics. In their active site, these hydrolytic enzymes contain either a serine residue (classes A, C, D) or a coordinated metal ion (Zn^{2+}) (class B) that mediates the hydrolytic cleavage of the β-lactam ring [15]. Since the discovery in the 1940s that pathogenic bacteria exposed to penicillin become resistant due to β-lactamase production, efforts immediately became focused on finding penicillin

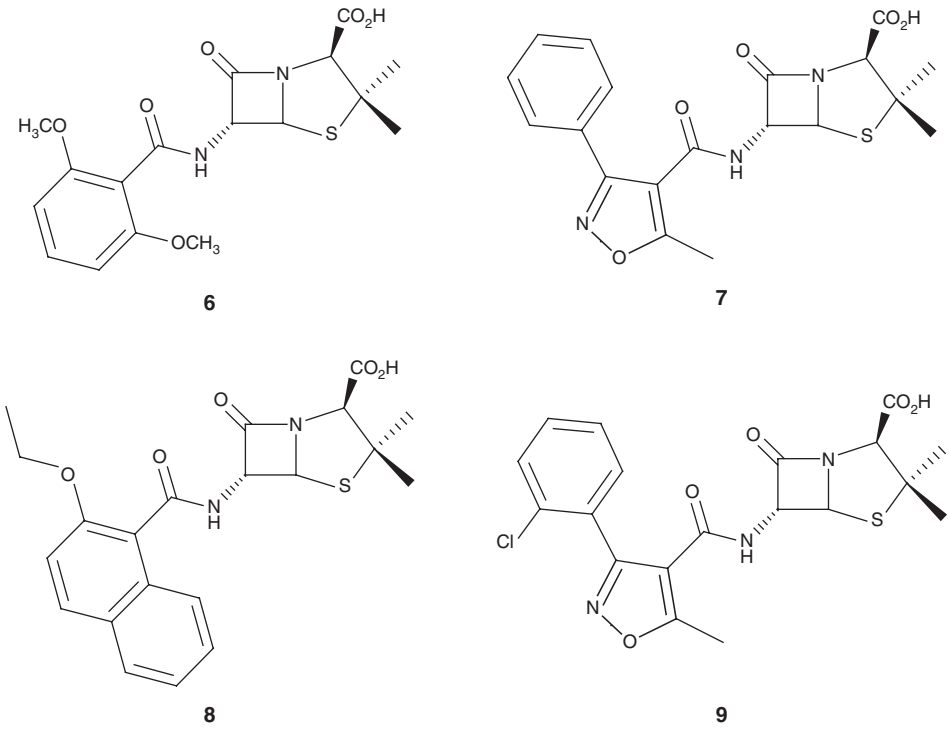


Figure 2. Structurally modified penicillins: methicillin (6), oxacillin (7), nafcillin (8) and cloxacillin (9).

analogs that could either resist hydrolytic cleavage or inhibit the catalytic activity of β-lactamases. The modification of the structure of the penicillin framework so that strong bactericidal activity is retained, but sensitivity to β-lactamase cleavage is diminished, was the first approach devised [16]. The structurally modified penicillins, including methicillin, oxacillin, nafcillin and cloxacillin (**Figure 2**), rapidly led to the evolution of new pathogenic bacteria, having β-lactamase-mediated resistance [17,18]. Although the development of cephalosporins and carbapenems in the late 1960s likewise yielded promising derivatives with improved antimicrobial profiles, resistance to these agents was also quick to develop [19,20]. Efforts were next directed at identifying inhibitors for the β-lactamase proteins, with the strategy of using a compound similar in structure to the antibiotic that can be readily recognized by the enzyme active site, but which is capable of irreversibly shutting down the catalytic cycle of hydrolysis. One approach consisted of screening microorganisms for the production of natural β-lactamase inhibitors [21]. In the early 1970s, the search turned out to be successful, with the discovery of the olivanic acids [22] and clavulanic acid [23], metabolites produced by *Streptomyces olivaceus* and *Streptomyces clavuligerus*, respectively. While clavulanic acid was a poor antibiotic, it was a very potent inhibitor of plasmid-mediated β-lactamases of both Gram-positive and Gram-negative species, and some chromosomal β-lactamases of *Klebsiella pneumoniae*, *Proteus* spp. and *Bacteroides fragilis* [10].

Since then, a vast number of structurally diversified β-lactam molecules have been isolated from bacteria or synthesized in the laboratory that have been shown to resist β-lactamase hydrolysis. Examples are the cephalosporins with a C7 β-oximino group (ceftazidime, cefotaxime, cefepime), cephamycins with a C7 β-methoxy group (cefoxitin), monobactam (aztreonam), carbapenem (imipenem) or suicide inhibitors, such as the penicillin sulfones (sulbactam, tazobactam and its derivatives) [10]. The combined use of β-lactamase inhibitors with a β-lactam antibiotic has been a successful method to combat resistance and recover the antimicrobial activity of the antibiotic [24,25]. Mechanism-based inactivators (clavulanic acid, sulbactam and tazobactam) have been brought to the clinic in combination with penicillins and are commercially available in amoxicillin-clavulanate, ticarcillin-clavulanate, ampicillin-sulbactam and piperacillin-tazobactam [10,26]. Thus, β-lactamase inactivators have extended the spectrum of activity of susceptible penicillins and cephalosporins to include β-lactamase-producing strains. Labile penicillins, such as ampicillin, in combination with sulbactam, or alternatively, amoxicillin with clavulanic acid, can now be used to treat infections caused by staphylococci and most class A plasmid-mediated β-lactamase producers. These inhibitors are not, however, active against all β-lactamases, and the AmpC chromosomal enzymes that are hyper-produced by some enterobacteria and pseudomonas are resistant. Moreover, genes for these AmpC enzymes have begun to escape to plasmids. Consequently, there is a clinical

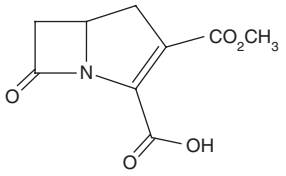


Figure 3. A carbapenem β-lactamase inhibitor.

need for more potent, broader-spectrum β-lactamase inhibitors [10]. In addition, with the frequency of clinical administration of antibiotic/inhibitor combinations, as well as the high reproduction rate and mutational frequency of bacteria, it is not surprising that further resistance has developed towards the β-lactamase inhibitors [27]. Such resistance may occur through overproduction of unmodified β-lactamase [28-30], by selective alteration of outer membrane proteins [31], as well as through generation of a mutant form of the β-lactamase, which is more resilient against the commercial inhibitors [32-35]. It seems that whatever we throw at the bacteria, they quickly adapt by developing resistance, thus highlighting the need for more innovative approaches, which can provide longer-term solutions to this ever-growing problem. One of the more recent areas of investigation towards this goal lies in the development of novel platforms for delivering and improving the effectiveness of β-lactam drugs. This review discusses the various strategies investigated so far to overcome β-lactam resistance, ranging from chemical modification of the antibiotic compounds to improve bioactivity or stability to penicillinases or alter the mechanism of action, to design of novel prodrugs that penetrate cells and deliver the antibiotic more efficiently, to use of drug delivery vehicles such as liposomes and nanoparticles to hide and protect sensitive β-lactam antibiotics from degradation.

2. Prodrug delivery of β-lactams

For a β-lactam antibiotic used in combination with a β-lactamase inhibitor to reach the target sites in bacteria, the journey starts (in the case of Gram-negatives) with their crossing the outer membrane of the target bacterium by passive diffusion through channels formed by the porin proteins [36]. These channels do present some barrier to free access to the periplasm (space between the outer cell membrane of Gram-positive bacteria and the cell wall). After penetration through the outer membrane, the antibiotic and the inhibitor must cross the periplasmic region on their way to the PBPs in the plasma membrane. Problems could arise along the way should the two molecules not approach and enter the same cell within a short period of time, a given likelihood if the penetration and migratory ability of the two molecules are significantly different. One conceptually simple approach is to use a dual-action agent that inhibits both the β-lactamase proteins and the targeted PBPs. An example of this was discovered for the naturally occurring β-lactam carbapenem (**Figure 3**).

Alternatively, tethering the β-lactam antibiotic directly to the β-lactamase inhibitor as a single deliverable molecule may enhance cellular uptake and ensure that both entities end up at the targets at the same time, provided that nothing acts on the compound along the way. This hybridized, dual-action molecule can be in the form of a prodrug, that is, a biologically inactive molecule that is converted to an antibiotic on reaching its target site. This strategy has potentially several key advantages over just using the active form of the drug itself, such as lower degradation and longer circulatory half-lifes. Prodrugs composed of a β-lactamase inhibitor (e.g., clavulanate) coupled covalently to a penicillin or cephalosporin antibiotic have been described [37], in which interaction of the clavulanate moiety with the β-lactamase first inhibits the hydrolytic enzyme and releases the penicillin/cephalosporin antibiotic by subsequent ring fragmentation (**Figure 4**).

If the bacterium carries the gene for the synthesis of a β-lactamase, then the periplasm may contain several thousand copies of this enzyme, which is no match for either the inhibitor or the antibiotic. Consequently, a change in strategy is mandated for the killing of such highly-resistant forms of bacteria that express β-lactamase. Moreover, certain β-lactamase inhibitors (e.g., clavulanic acid and sulbactam) have been found to actually further induce β-lactamase expression in the microbes they act on, which diminishes their effectiveness [38,39]. Enzyme-catalyzed therapeutic activation (ECTA) is another prodrug delivery strategy to overcome β-lactam drug resistance resulting from the overexpression of β-lactamase proteins. Based on the ECTA targeting concept, the β-lactamase can in effect be used as a biological sensor to generate antibacterial agents in drug-resistant microorganisms by coupling a cytotoxic agent onto a β-lactam framework. This delivery design ensures that the β-lactamase proteins cause the release of the active agent directly to the resistant bacterial cell. Examples of this are NB-2001 and NB-2030 (**Figure 5**), recently described cephalosporins that release the biocide triclosan following β-lactam ring cleavage. Each of these compounds demonstrates antimicrobial activity against β-lactamase-producing strains of several Gram-negative bacteria [40,41].

Another example is sultamicillin, a chemical conjugate of sulbactam (a semisynthetic β-lactamase inhibitor) and ampicillin (a penicillin antibiotic), which extends the activity of ampicillin to β-lactamase-producing bacteria. Ro-23-9424 acts similarly, having a β-lactamase-sensitive cephalosporin hooked onto a fluoroquinoline, which is the active antibiotic (**Figure 6**) [42].

A new class of dimeric β-lactams (carbapenem prodrugs) that target both β-lactamase and transpeptidase has also been reported (**Figure 7**) [43].

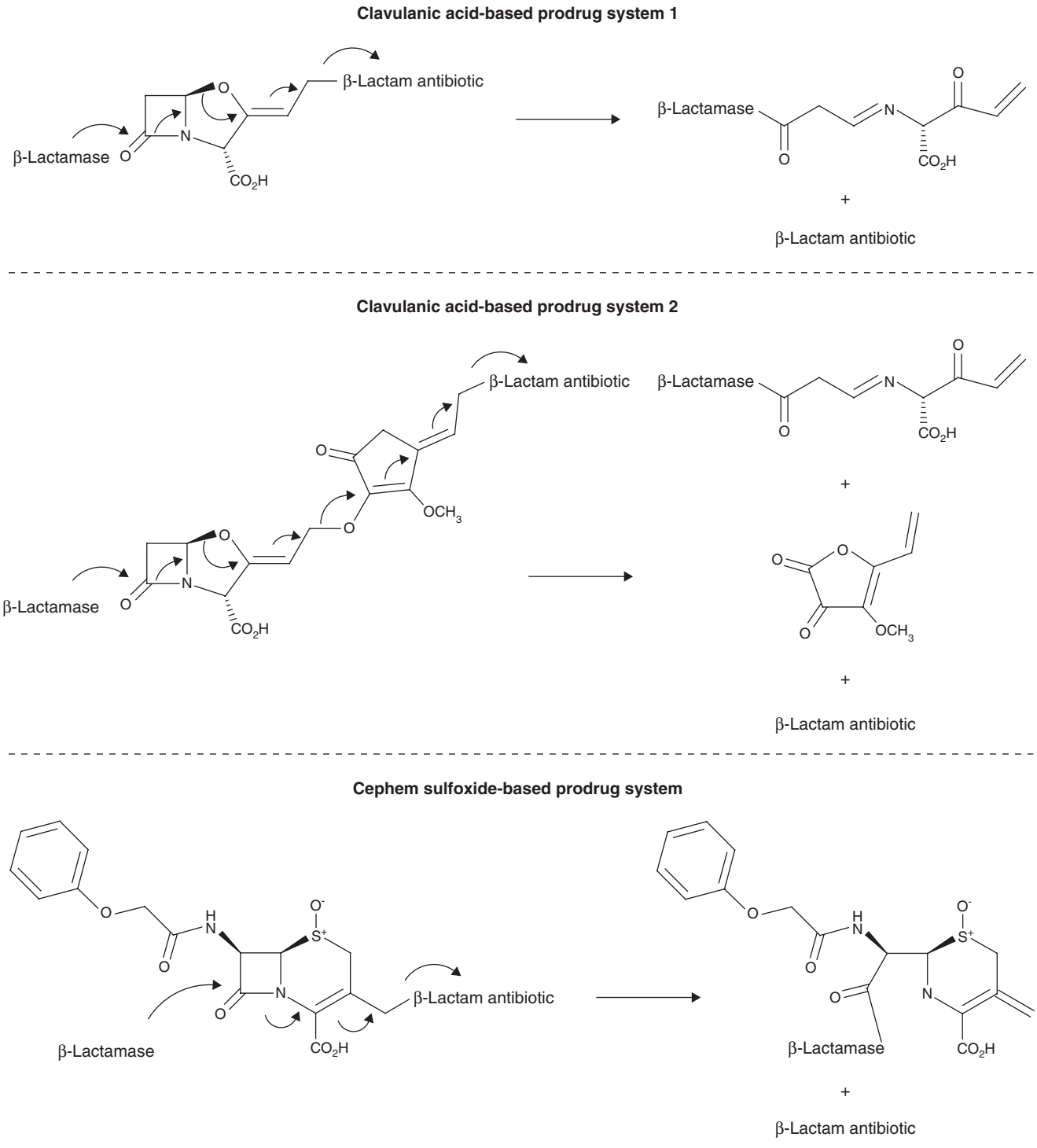
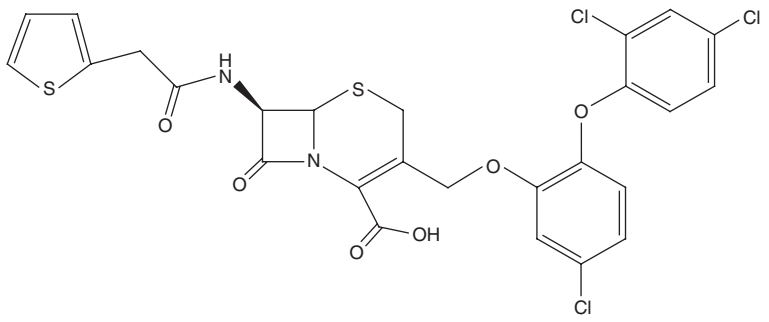
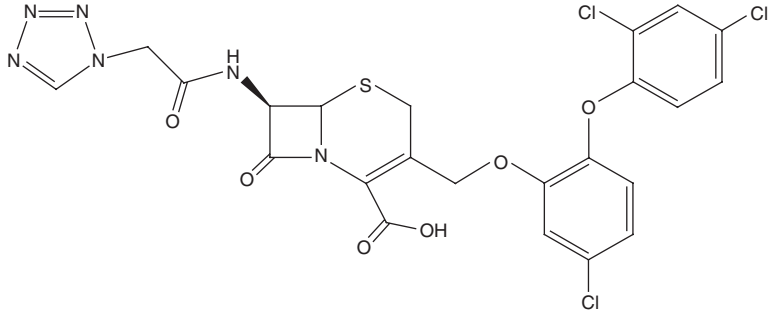


Figure 4. Mechanism of three different dual-action prodrug delivery systems that release an active β-lactam antibacterial only in the presence of β-lactamases.

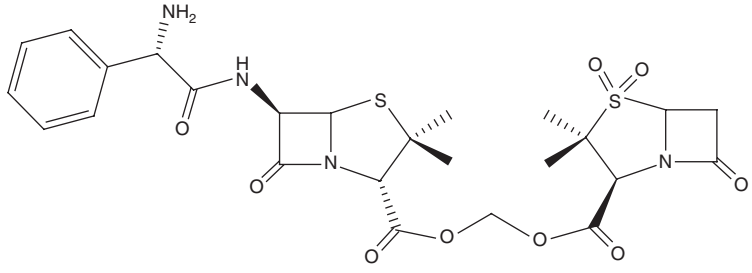


NB2001

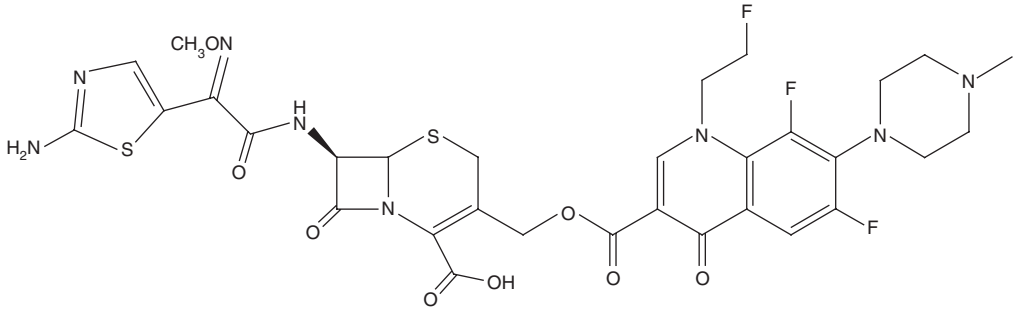


NB2030

Figure 5. Cephalosporin–triclosan dual-action drugs.



Sultamicillin



Ro 23-9424

Figure 6. Sultamicillin and Ro-23-9424.

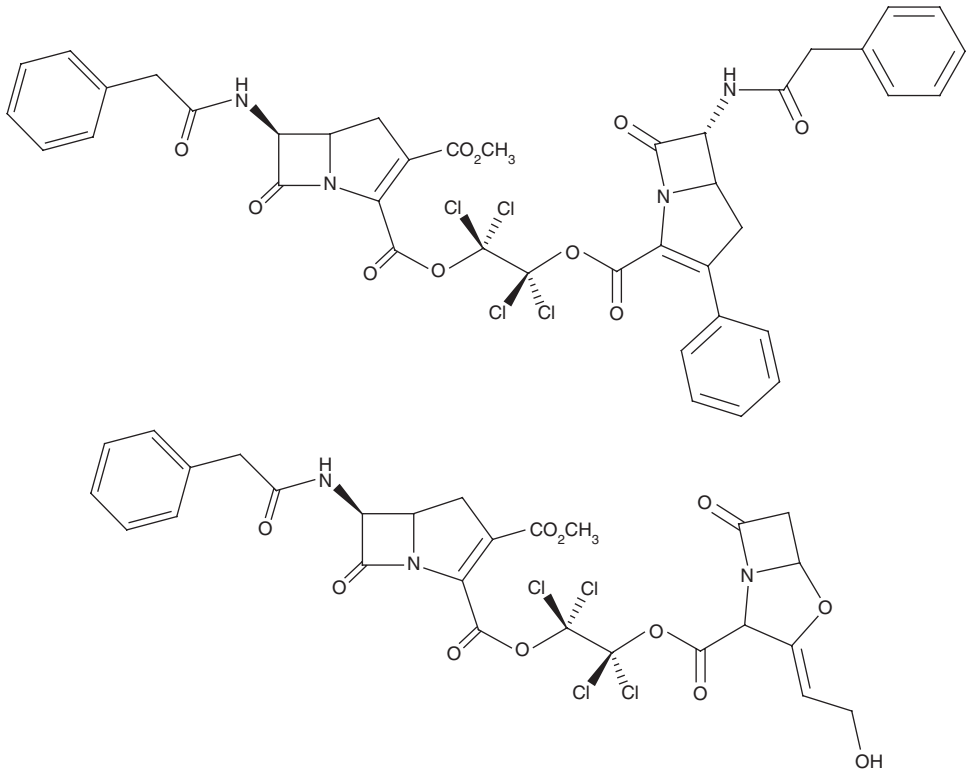


Figure 7. Dimeric carbapenems (top) and a clavulanate derivative of carbapenem (bottom).

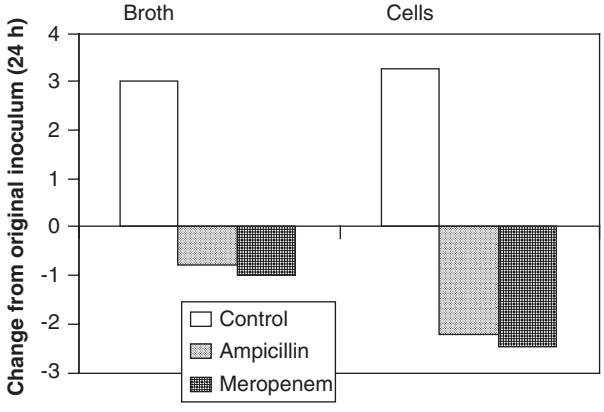


Figure 8. A comparison of the antibacterial activity of two representative β-lactam antibiotics (ampicillin, meropenem) against extracellular (broth) and intracellular (THP-1 macrophages) forms of *L. monocytogenes* at 50 µg drug/ml in a 24-h model. The paradox here is that both β-lactams are more active in infected cells than in broth.

3. Delivery to intracellular bacteria

One of the more recently recognized types of antibiotic resistance is that caused by intracellular pathogens, which provide a reservoir for recurring infections. These occur when microbes enter mammalian cells such as macrophages to take up residency and remain in a state of dormancy, which shelters them from the cidal effects of antibacterial drugs. There is now substantial evidence indicating that the intracellular milieu may in fact modulate both the pharmacological properties of the antibiotics and the response of the bacteria to those drugs [44]. For instance, both ampicillin and meropenem kill intracellular bacteria more rapidly and more extensively than the corresponding extracellular bacteria (**Figure 8**) [45].

In the course of a second study on the activities of β-lactams against *S. aureus* phagocytosed into human THP-1 macrophages, it was noted that MRSA and methicillin-sensitive *S. aureus* strains showed similar susceptibilities to meropenem, thus suggesting a restoration of susceptibility of MRSA to β-lactams in the intracellular milieu [46,47]. This finding raises the question as to whether β-lactam drugs that are determined to be ineffective against MRSA *in vitro* can still remain highly effective against intracellular forms of MRSA (and other bacteria). There is evidence suggesting that this surprising restoration of susceptibility of intra-phagocytic MRSA to meropenem and cloxacillin is likely to be due to the acidic pH prevailing in the vacuoles where *S. aureus* resides, which enhances the binding of the drugs (in its protonated carboxylic acid form) to PBPs [48]. The studies, however, describe diminished accumulation (i.e., an apparent intracellular concentration lower than the extracellular one at equilibrium) for β-lactam antibiotics

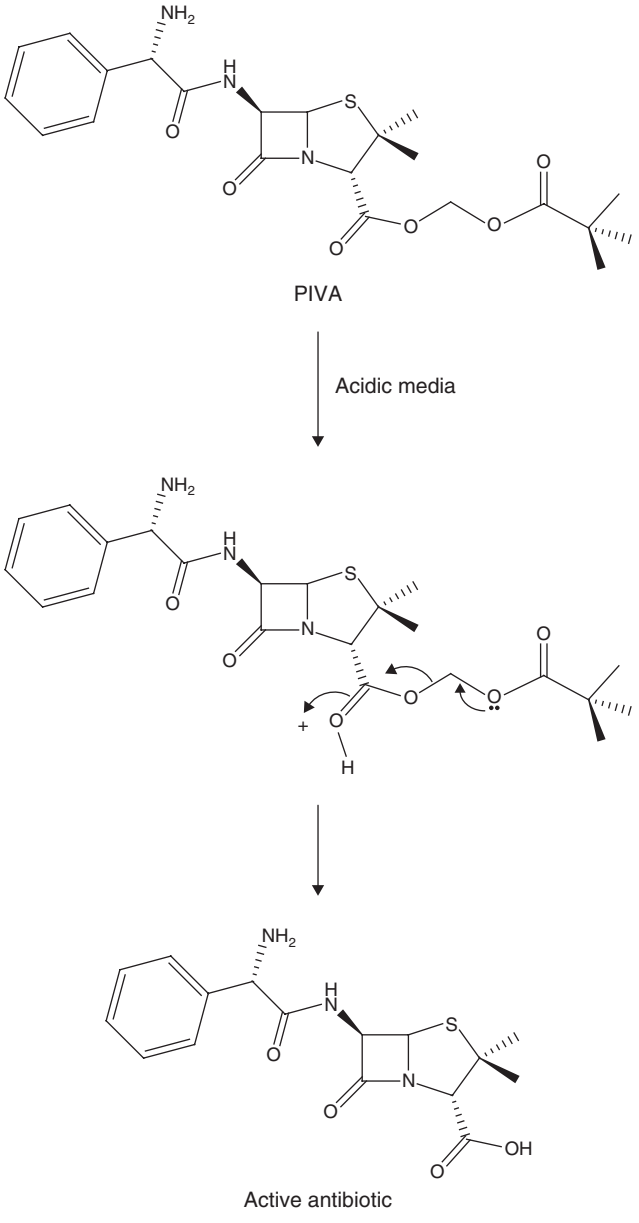


Figure 9. Acid-promoted cleavage of pivaloyloxymethyl ampicillin.

whether in phagocytic or non-phagocytic mammalian cells and tissues in general.

Neutrally-charged β-lactam prodrugs have thus been designed and evaluated in order to increase the cellular accumulation of the antibiotic. Pivaloyloxymethyl ampicillin (PIVA; pivampicillin) (**Figure 9**) is an esterified derivative of ampicillin that has been demonstrated to be delivered into cells as a prodrug [49], where the free carboxylic acid form of the active antibiotic is generated intracellularly by acid-promoted cleavage of the ester bond. PIVA was found to be active against intracellular *Listeria monocytogenes*, even when β-lactamase was added to the extracellular milieu. This indicates that the conversion of PIVA to ampicillin must take place, at least partially and as far as intracellular activity is concerned, in a place where ampicillin will be shielded from the extracellular milieu.

4. Delivery using drug carriers and nanoparticles

Various types of drug delivery strategies have also been explored with different classes of antibiotics (including β-lactams), for the purpose of improving therapeutic efficacy through more favorable drug bioavailability, serum stability and pharmacokinetics [50]. The basic premise is a very simple one conceptually: to encapsulate or physically protect the drug from the outside environment within the matrix of some sort of delivery scaffold. Options for this are quite diverse, although most of the methods studied have not been implemented beyond *in vitro* experiments. Thus, there remains a wealth of things to do to bring these methodologies to fruition, and hurdles to overcome with each. Moreover, although these approaches may have never been specifically designed to overcome β-lactam drug resistance mechanisms per se, this possibility certainly exists, and thus is relevant to the discussion.

The first drug delivery systems developed in the early 1970s consisted of microspheres and microcapsules that allowed drugs to be placed inside, and then implanted as close to the area of infection as possible so as to release the active drug near the target site [51,52]. This was then followed with much smaller colloidal particulates less than a micron in size, such as liposomes, nanospheres and nanocapsules, that had greater mobility in the body and thus better ability to actually carry, or deliver, an antibiotic to the infection [53]. It was found, however, that after intravenous administration most colloidal particles are quickly scavenged by phagocytic cells in the liver and spleen, depending on their size and surface characteristics, and concentrated there for elimination [54]. The early particulates thus had limited utility as drug carriers due to rapid clearance from the body as a result of plasma proteins depositing onto the surface, and subsequently recruiting phagocytes for disposal by the reticulo-endothelial system (RES). Stealth systems then came about by modifying the surface of the particles with surfactants that shielded the surface from these proteins, thus greatly enhancing the serum lifetimes. This allowed for a longer period of controlled drug release [55]. Finally, target-seeking drug carriers were devised, such as monoclonal antibodies [56], as well as liposomes and nanoparticles tethered to antibodies or to other recognition elements [57], enabling specific recognition of a cellular target by the carrier [58,59]. Nanoparticles consisting of organic polymers, cyclodextrins [60] or metals, allow the bioactive molecule to reside either within or on the surface. So far, the vast majority of studies using these types of drug carriers have been for targeted drug delivery to cancer cells; however,

antibiotics have also been studied in some cases (including β-lactams), as summarized in the following discussion.

Liposomes have been studied as carriers of biologically-active compounds since 1971 and evaluated intensively across various disease categories for drug delivery applications [61]. Liposomes consist of phospholipid bilayers with an aqueous phase in the center due to the hydrophilic heads of the liposome organizing towards the bulk aqueous media, and the hydrophobic tails lining up inwards. In water, this creates an unusual bilayer wherein a water-soluble drug such as penicillin can be entrapped inside the aqueous center, and lipophilic compounds can concentrate within the lipophilic bilayer structure itself. How liposomes function as delivery vehicles depends on the type of cells they interact with, but the premise is that delivery of an antibiotic occurs through endocytosis, which follows the initial interaction of the liposome with the cell membrane, either through a mediated or passive fusion of the lipoproteins, which releases the encapsulated drug. One of the drawbacks of liposomes is their stability, which may be difficult to control in terms of programmed drug release. Pegylated variants now exist that provide stealth properties and much longer serum lifetimes. Liposomes, thus, are highly suitable for antibiotics delivery.

Hydrogels consist of polymer strands such as poly(vinyl alcohol) that spontaneously form colloids in water [62]. As for liposomes, antibiotics can be entrapped inside the crosslinked matrix and be released in a controlled manner [63]. A few applications of hydrogels as delivery vehicles for antibiotics have been reported and studied *in vitro* [64]. However, as yet, β-lactams have not been investigated in this context.

Nanoparticles are another major category of drug delivery vehicles for a wide assortment of drug types. By definition, these carriers may have dimensions of 1 – 1000 nm, but most of those reported in the literature are generally 5 – 350 nm in diameter, and can be made from essentially any type of biocompatible substance [65]. The most common are polymeric systems that form nanospheres in aqueous media. Many types of lipophilic drugs and water-soluble drugs, including antibiotics, can be carried inside or on the surface of the nanoparticle, either through covalent attachment to the nanoparticle matrix, or by encapsulation or surface association [66]. Nanoparticles are typically more stable than liposomes in biological fluids and during storage. Various methods for producing drug-carrying nanoparticles have been developed, including spray drying and ultra-fine milling [67], which give broad distributions of particle sizes, nanoprecipitation [68], and emulsion polymerization [69]. As a class, nanoparticles can have favorable pharmacological properties for drug delivery due to their biocompatibility, biodegradability, consistent morphology and uniquely small size [70]. Different tissues and organs respond to nanoparticles of specific size ranges. Nanoparticles <100 nm in size can avoid being recognized by the RES and get into bone marrow, whereas those > ~ 300 nm are quickly picked up by phagocytes (except for the stealths) and are not able to penetrate into heart and lung tissue [71,72]. Nanoparticles emulsified in water can usually be delivered orally and be readily absorbed in the gastrointestinal tract, where the nanoparticle protects the drug from degradation while inside [73]. Once absorbed, the nanoparticles can serve as a drug reservoir in releasing the antibiotic over a prolonged period. This certainly has implications for overcoming drug resistance mechanisms in that the nanoparticles can navigate the sensitive β-lactam molecules through the regions around the resistant bacteria where β-lactamases conduct surveillance. Nanoparticles can also be administered through intravenous, subcutaneous and intraperitoneal injection, which may have advantages depending on whether the resistant infection is localized in skin, certain tissues, or is systemic. The most relevant nanoparticles for antibacterial applications so far include polyacrylates, polylactide-co-glycolides and metal nanoparticles.

Polymeric nanoparticles have been studied as drug carriers since the 1970s, with Kreuter and Speiser showing the use of polyacrylamide nanoparticles forming in the presence of antigens that led to a novel vaccine adjuvant [74]. Three years later, Couvreur's laboratory reported on a series of poly(alkyl cyanoacrylate) (PACA) nanoparticles that are bioresorbable, and are being used as surgical glues [75]. Couvreur developed and investigated in detail PACA nanoparticles over a 30-year period, and has studied their use extensively with a wide variety of drugs (including antibiotics) both *in vitro* and *in vivo*. An outstanding comprehensive review of this topic was recently published [76]. Poly(alkyl cyanoacrylate) nanoparticles are prepared in water in the presence of the antibiotic at pH 3 by emulsion polymerization [77]. The procedure requires the use of surfactants to prevent aggregation and to control a uniform particle size distribution [78]. Couvreur also synthesized poly(isobutyl cyanoacrylate) nanoparticles for the entrapment of ampicillin. These nanoparticle emulsions can be freeze-dried to remove the water, and then reconstituted as needed. Thus, the nanoparticles are very stable in storage as dried powders. The nanoparticles depend on the amount of drug loading, ranging from around 130 nm for no drug, to about 200 nm for 2 mg/ml of loaded ampicillin. Freeze-drying does not affect nanoparticle size or properties. Efficiency of drug loading into the nanoparticle matrix, and subsequent drug release, both depend on the type of nanoparticle matrix used. Interestingly, encapsulation of ampicillin into poly(isohexyl cyanoacrylate) nanoparticles significantly enhances efficacy of the drug by 120-fold in treating intracellular infections in mice [79]. For instance, mice infected to *Salmonella typhimurium* responded to merely 0.8 mg of ampicillin bound to the nanoparticles in the same way as three 32-mg doses of ampicillin itself. Similarly, these nanoparticles increased bioactivity of the β-lactam by 20 times in treating *L. monocytogenes* infection. Ultrasonic autoradiography indicated that the nanoparticles diffused

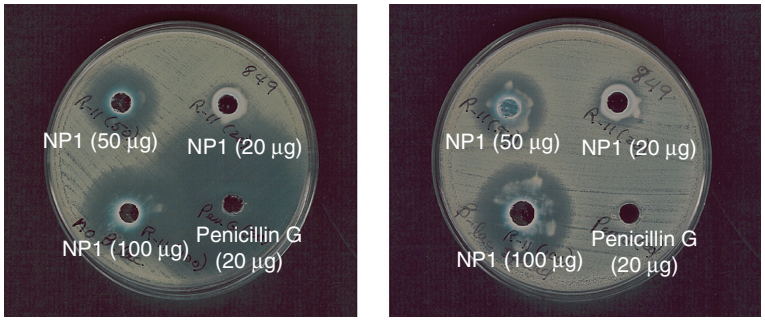


Figure 10. Kirby–Bauer studies of penicillin-conjugated polyacrylate nanoparticles against *S. aureus* (ATCC 25923).

through the human cell to target the cell wall of the bacteria living inside [80]. This enhancement is thought to be due to not only improved cellular uptake of the water-soluble antibiotic by the nanoparticle, but also to an increase in concentration of drug by capture of the nanoparticles in the liver and spleen. Thus, these nanoparticles help in the delivery of β-lactams to intracellular bacteria, especially those in the RES organs. Couvreur also demonstrated that other types of antibiotics such as ciprofloxacin (cipro) can be incorporated into these nanoparticles, and that they possess significantly higher antibacterial activity against *Mycobacterium avium* complex in human macrophages than the free cipro. The ability to introduce a diverse selection of different antibacterial classes into these nanoparticles of course raises the possibility of multi-drug containing systems for overcoming drug resistance to β-lactams.

Fontana's group has likewise studied poly(ethyl cyanoacrylate) (PECA) nanoparticles as a means to entrap β-lactam antibiotics such as ampicillin in aqueous media [81]. For surfactants, Fontana employed pluronics (non-ionic polyoxyethylene-polyoxypropylene block co-polymers) of different molecular weight ranges, and the average sizes of these nanoparticles are around 350 nm. Different drug concentrations do not alter the size of the particles. The particles are stable at physiological pH for at least 5 h, but rapidly degrade in acidic media. Release of ampicillin is thus dependent on the pH and the surfactant structure, with quicker release occurring for less hydrophilic and larger molecular weight pluronic surfactants. The hydrolysis of the ester side chain of the poly(alkyl cyanoacrylate) is the main degradation mechanism, which can be catalyzed by esterase hydrolysis at pH 7.4. Antimicrobial activity of PECA nanoparticles was found to be equal to or greater than that of free ampicillin, but studies against resistant microbes have not been reported. Fontana later introduced amoxicillin-loaded PECA nanoparticles, which are surface coated with polyethyleneglycol using pluronic F68 [82]. For these, particle size is dependent on the molecular weight of the PEG additive, but is generally 200 – 320 nm. These PECA nanoparticles may be useful for site-specific delivery of β-lactams to the stomach. Uptake by phagocytes is greatly reduced for the PEG-coated (stealth) nanoparticles compared with non-pegylated systems, thus increasing dramatically the serum half-life and drug carrier capabilities.

Polymer-based nanotechnologies have also been demonstrated to rejuvenate antibacterial activity of penicillin in the presence of β-lactamase, thus afforded strong antibacterial activity against methicillin-resisant *S. aureus* [83]. Penicillin-containing polyacrylate nanoparticles, prepared by free radical emulsion polymerization in water using either acrylated penicillin monomers (for covalent attachment to the nanoparticle matrix) or penicillin esters (for encapsulation into the nanoparticle), retain their full antimicrobial properties even in the presence of high concentrations of β-lactamase. This effect is illustrated in the microbiological experiment shown in **Figure 10**.

Microbiological assays performed against a penicillin-susceptible strain of *S. aureus* in the absence of added penicillinase protein (left image) versus in the presence of 100 μg of penicillinase added to the agar (right image) compare the effects of the nanoparticle. In this experiment, penicillin G lost all of its antimicrobial activity when the penicillinase is added to the media, while the penicillin nanoparticles fully retain their activity at all three drug amounts, as noted. Moreover, this *in vitro* bioactivity of these nanopenicillins was found to be closely dependent on both the structure of the penicillin derivative used to make the nanoparticle, as well as on the type of functionality linking the penicillin to the polymeric framework. The order of *in vitro* bioactivity observed in the assays follows the ease of cleavability of the penicillin molecular from the polyacrylate chain, with imide > ester > amide.

Ludwig synthesized cipro-loaded poly(lactic-co-glycolide) (PLGA) nanoparticles measuring ~ 200 nm in size by high-pressure homogenization [84]. These nanoparticles were examined against *S. aureus* and *Pseudomonas aeruginosa* and found to be at least as active as the free drug. No studies have yet been reported for penicillin-containing variants or against β-lactamase-producing strains of bacteria, such as MRSA.

However, gentamycin sulfate, an ionic antibiotic, could be loaded into PLGA nanoparticles, and these have been

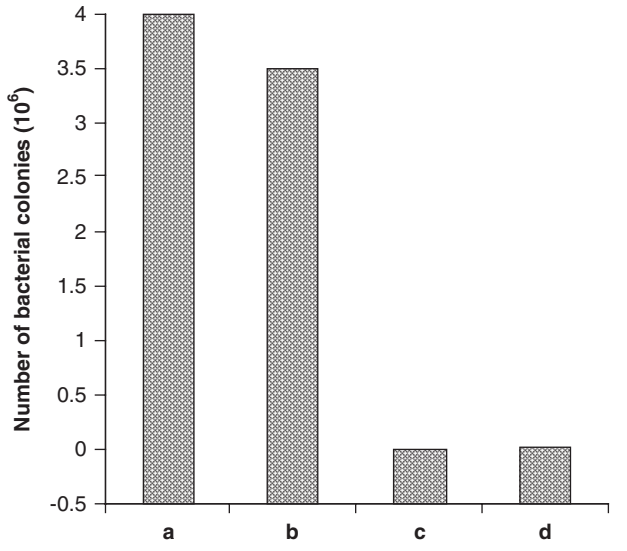


Figure 11. Effect of amoxicillin and nanosilver on *E. coli*., individually and jointly, on 5 × 10^6 bacterial colonies. a) 5 µg/ml nanosilver; b) 150 µg/ml amoxicillin; c) 150 µg/ml amoxicillin plus 5 µg/ml nanosilver; d) 150 µg/ml amoxicillin plus 10 µg/ml nanosilver.

found to possess good activity against *S. aureus*. It is believed that this may be useful for treating intracellular *Brucella* infections due to the likely uptake by phagocytes [85].

Emulsions made of benzathine benzylpenicillin G-containing PLGA nanoparticles can be formed by spontaneous (or self) emulsification, and these structures measure ~ 200 nm in size [86]. This gives very high (85%) efficiency for encapsulation of the drug, which can be released systemically within about 2 h.

Metal nanoparticles have also been explored for drug delivery applications, including for DNA, proteins, and antibiotics [87,88]. The ability to easily form small (~ 5 nm), uniformly shaped structures makes them attractive as drug carriers, where the antibiotic is appended to the surface via surface charges or thiol linkages.

Silver nanoparticles are widely recognized as excellent antimicrobial agents because of their powerful biocidal ability and non-toxicity to human cells [89-91]. The possible mechanism of killing microorganisms by silver ions may be explained as follows: i) silver ion inhibits ATP synthesis via binding to the ATP synthesis enzyme molecules in the cell wall; ii) silver ion enters the cell and binds with DNA, leading to the DNA denaturation; and iii) silver ion blocks the respiratory chain of microorganisms in the cytochrome oxidase and NADH-succinate-dehydrogenase region [92]. It has been reported that the mode of antibacterial action of silver nanoparticles is similar to that of silver ion [93]. However, the effective biocidal concentration of silver nanoparticles is at a nanomolar level in contrast to a µmolar level of silver ions, so clearly there is more to this correlation. In addition the antimicrobial activity of colloidal silver particles can be influenced by the dimensions of the particles, with the smaller the particles, the greater the antimicrobial effect. Recently, Panacek *et al.* showed that silver nanoparticles of average size of 25 nm have strong antimicrobial and bactericidal activity against both Gram-positive and Gram-negative bacteria, including multi-drug resistant microbes such as methicillin-resistant *S. aureus* [94]. Silver nanoparticles combined with poly(methyl methacrylate) nanofiber composites have been applied as antibacterial materials from the viewpoint of releasing silver nanoparticles and possessing 'on contact' biocidal activity. The antibacterial kinetics and minimum inhibitory concentration test showed that the silver/polymer nanofiber have a three-fold killing rate and antimicrobial activity greater than $AgNO_3$, and nine times that of silver sulfadiazine [92]. Importantly, the polymer nanofiber showed excellent biocidal potential against Gram-positive bacteria (*S. aureus*) as well as Gram-negative bacteria (*Escherichia coli*).

The antimicrobial effects of combining β-lactam and silver nanoparticles have been described [95]. The combination of nanosilver and amoxicillin has a synergistic effect on antibacterial efficiency against *E. coli*., as shown in **Figure 11**. The bars corresponding to nanosilver and amoxicillin alone (**a**, **b**) show little individual activity, whereas bars **c** and **d** indicate essentially total destruction of bacterial colonies when the two are used together.

Amoxicillin molecules contain a number of reactive functional groups such as hydroxy and amido groups, which can react easily with nanosilver by chelation, and may make up a nanoparticle sphere in which a nanosilver core is surrounded by chelated amoxicillin molecules as shown in **Figure 12**.

As discussed above, silver nanoparticles (nanosilvers) combined with amoxicillin can kill bacteria via three completely different mechanisms, and thus provides a promising alternative strategy to overcome antimicrobial resistance to the β-lactams. It was also found that cefoperazone (**Figure 13**), a third-generation semisynthetic cephalosporin antibiotic, is antibacterially synergistic against MRSA if used in conjunction with colloidal silver (**Table 1**) [96]. These studies illustrate that synergistic pairing of a β-lactam drug with other adjuvants or delivery materials can enhance antibacterial activity overall.

Xu reported vancomycin-coated gold nanoparticles that showed activity against vancomycin-resistant enterococci [97]. A similar construct has been reported for ciprofloxacin-coated gold nanoparticles [98]. It was noted that gold nanoparticles surface modified with cationically charged side chains are somewhat toxic, whereas the anionic variant is not, and this is an important reminder of the effects of surface charge on cellular toxicity [99].

Porous hollow silica nanoparticles have been briefly investigated as drug carriers due to their extremely high chemical and thermal stability, and huge surface area for drug adsorption. These unusual nanoparticles are

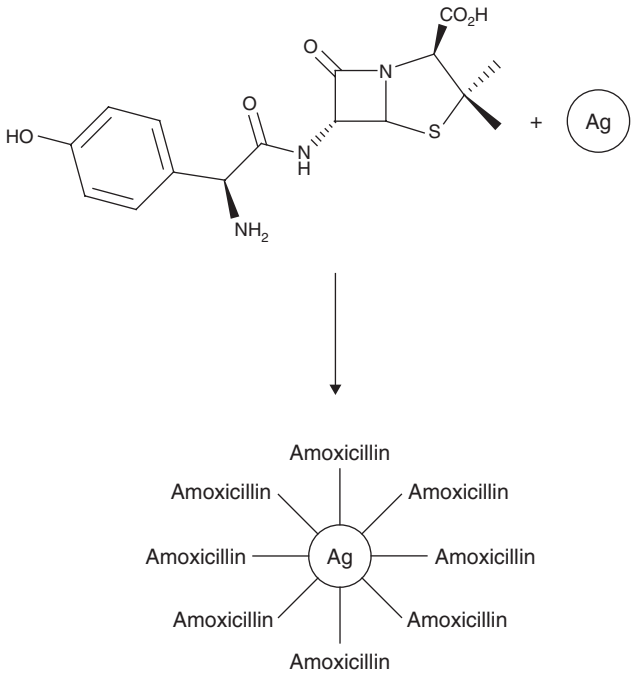


Figure 12. Silver nanoparticles chelated with amoxicillin.

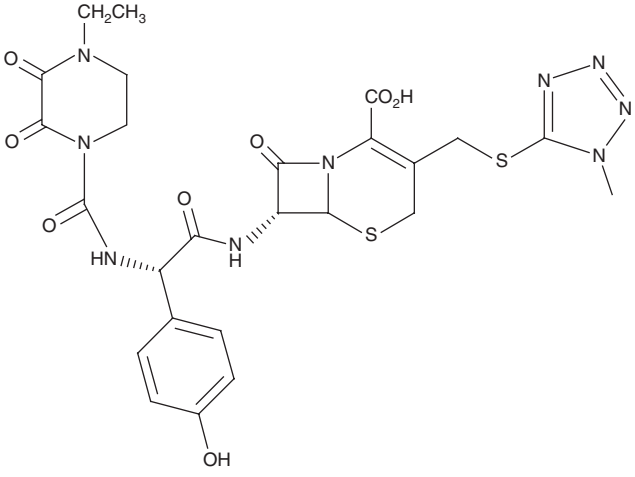


Figure 13. Cefoperazone.

made by using calcium carbonate as a template for the silica lattice to form as an aqueous suspension, which when evaporated produce spherical nanoparticles with a diameter of 60 – 70 nm [100]. The outer wall of the silica particle itself is about 10 nm thick. The cephalosporin, cefradine, has been used to coat the inside surface crevices of the porous silica particles. Interestingly, the pores release drug rapidly (~ 74% drug is released in 20 min), which then slows. Attempts to use β-lactams or to devise controlled delivery for drug-resistant bacteria has, as of yet, not been reported.

The interaction of nanoparticles with human tissue as well as microorganisms is an expanding field of research, one which as yet is largely unexplored within the realm of antibacterials delivery and treatment of infectious diseases.

5. Other approaches

The permeability factor controlling access of the antibiotic to its cellular target is often critical in determining the efficacy of β-lactam drugs against a Gram-negative bacterium, where expression of porin OmpF and OmpC plays an important role in antibiotic resistance [101]. Hence, strategies aimed at outer membrane barriers can be effective at reversing antimicrobial resistance in these organisms. Clinical isolates of *Shigellae*, which are lacking or have mutated porin OmpF, were resistant to a number of β-lactam antibiotics. It has been reported that the aqueous extract of plant *Aegle marmelos* (AEAM) influences susceptibility of β-lactam-resistant *Shigella* toward β-lactam antibiotics such as ampicillin, penicillin and carbenicillin by altering porin channels [102]. The synergistic effect of AEAM on β-lactam-resistant clinical isolates of *Shigella dysenteriae* and *Shigella flexneri*, which increases susceptibility toward β-lactam antibiotics, shows that this kind of combinational therapy can be used for treating multi-drug resistant bacteria.

PBPs determine susceptibility and resistance to β-lactam antibiotics in all Gram-positive bacteria. β-lactamases are a significant and prevalent mechanism of resistance to β-lactams in both Gram-positive and Gram-negative bacteria. Novel β-lactams that are effective against these mechanisms have been reported. Ceftobiprole (**Figure 14**) is a major advance from existing β-lactams, with a high affinity for the altered PBP 2′ (2a), making it active against methicillin-resistant staphylococci. It also binds avidly to the relevant PBPs of most Gram-positive and Gram-negative pathogens, and is resistant to hydrolysis by many β-lactamases [103,104].

Ceftobiprole demonstrates potent binding to PBPs from Gram-positive bacteria, including those with decreased β-lactam sensitivity, such as PBP2a in MRSA and PBP2x in a penicillin-resistant *Streptococcus pneumoniae* strain. In *E. coli*, ceftobiprole exhibited strong binding to the essential PBPs PBP2 and PBP3. These binding profiles explain the broad-spectrum activity for ceftobiprole that includes Gram-negative bacteria and many β-lactam-resistant Gram-positive cocci, including MRSA. The activity of ceftobiprole against β-lactam resistant Gram-positive cocci can be attributed to the nearly equipotent inhibition of the normal complement of sensitive PBPs that are the killing target of β-lactam antibiotics, and of the β-lactam-insensitive PBPs, which are the principal determinants of high-level β-lactam resistance in these organisms. In staphylococci, it is an additional protein – PBP2a – that is the major cause of β-lactam resistance. Ceftobiprole reacts rapidly with this target, exhibiting high affinity and forming a stable inhibitory complex in which the ceftobiprole moiety makes multiple

Table 1. MIC values for colloidal silver, cefoperazone and their combinations against MRSA.

Bacterial strain	Cefoperazone MIC (μg/ml)	Colloidal silver MIC (μg/ml)	FIC of colloidal silver and cefoperazone combination
MRSA	10	0.5	0.18

The interaction between drugs was quantitatively evaluated by means of the FIC, which was calculated by the following formula:
FIC = [MIC A in combination/MIC A] + [MIC B in combination/MIC B]. The interaction was defined as synergistic if the FIC index was ≤ 0.5.
FIC: Fractional inhibitory concentration index; MIC: Minimum inhibitory concentration; MRSA: Methicillin-resistant *Staphylococcal aureus*.

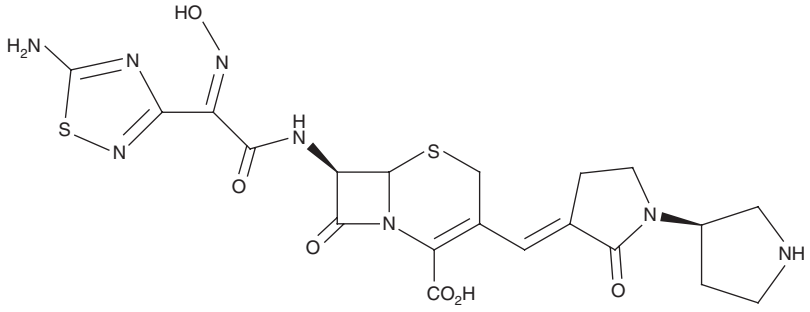


Figure 14. Ceftobiprole.

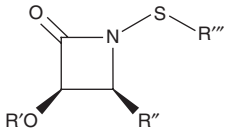


Figure 15. Structure of *N*-alkylthio β-lactams.

interactions with the protein. In addition, ceftobiprole is relatively stable towards class C β-lactamases.

The development of new β-lactam antibiotics with alternative mechanisms of action, such as *N*-alkylthio β-lactams (**Figure 15**) [105-107], offers an unprecedented new approach to tackle the resistance problem. This family of antibacterial compounds shows promising activity against only a few strategic pathogenic bacteria, a short list that includes MRSA and *Bacillus anthracis*. Despite any structural similarities to the penicillins and the monocyclic β-lactams such as the monobactams, these *N*-thiolated β-lactam compounds behave differently. Instead of inhibiting cell wall crosslinking proteins to afford broad-spectrum bacteriocidal activity against the Gram-positives, these *N*-thiolated lactams are bacteriostatic in their behavior, acting through a completely different mechanistic mode, and being uncleavable by penicillinases. These compounds react rapidly within the bacterial cell with coenzyme A (CoA) through *in vivo* transfer of the *N*-thio group to produce an alkyl-CoA mixed disulfide species, which then interferes with fatty acid biosynthesis through a yet undetermined pathway [108]. Therefore, effects on bacterial morphology or cell wall structure are not observed, but instead, rapid degradation and destruction of the cellular membranes occurs. These *N*-thiolated lactams have been incorporated into polyacrylate-styrene nanoparticles by radical-induced emulsion polymerization and found to have promising antibacterial properties against MRSA and *B. anthracis* [109,110].

6. Conclusions

For > 60 years, the β-lactams have played a major role in improving human health. As a class, the penicillins have been the workhorse in treating a wide assortment of bacterial infections ranging from minor sore throats to life-threatening systemic attack. The emergence of resistance to penicillin and its many variants worldwide poses a serious threat to its continued use, thus placing high demand for ways to recover and improve the effectiveness of this important class of antibiotics. Because of their prevalence as a major family of antibiotics, low cost and minimal side effects, β-lactams do remain useful for the treatment of human bacterial infections, but strategies have to be developed to preserve their antibacterial activity in the face of increasing bacterial resistance. In the early years, the major drug companies invested their resources to produce more powerful penicillin derivatives that are more resistant to β-lactamase hydrolysis while being more aggressive at targeting the penicillin binding proteins. This led to temporary success, but in the end, still greater resistance development. The introduction of β-lactamase inhibitors soon followed, allowing the delivery of penicillin to the target by blocking the destructive action of β-lactamase proteins. This likewise had success, in the beginning, but has succumbed to resistance. More recently, dual action

drugs and prodrugs, having a β-lactamase inhibitor chemically attached to the penicillin (or another antibiotic) have been explored as a means to release the drug into the resistant (β-lactamase-producing) bacterial cell. This approach suffers from having to chemically synthesize the agents, which is expensive and cannot yet be done on a commercial scale for worldwide distribution, and is still only at the exploratory stage. The recent work with intracellular bacteria has shown a surprising restoration of effectiveness for β-lactams against these pathogens residing within human cells, which raises hopes for the use of penicillin for treating dormant bacteria that cause recurring infections. However, delivery of these water-soluble antibiotics into the human cell is an issue, and so, drug delivery approaches are necessary. Nanoparticles have recently been studied as a means to deliver β-lactams, as well as to protect them from β-lactamases. These delivery vehicles may ultimately rejuvenate penicillin's activity by avoiding resistance mechanisms and helping to get the drug to where it really needs to go: to the site of infection. Numerous possibilities present themselves through combinations of these independent approaches, synergizing those that work for enhancing antimicrobial activity with those that can reduce the problems of drug resistance.

7. Expert opinion

It is so often heard in the popular media that the antibiotics era is over; that treating even the most mundane bacterial infections nowadays is becoming so difficult that we are facing a return to the preantibiotic, prepenicillin period. Is the golden age of penicillin and antibiotics in general really coming to an end? To be sure, there are several good reasons for pessimism. Humanity's ongoing war with microbes began in full force with penicillin during the end of World War II in the early 1940s, and at that time victory seemed certain. The consensus was that infectious diseases would soon be a thing of the past thanks to the wonder drug penicillin. However, even penicillin's discoverer, Sir Alexander Fleming, strongly contested this view and in accepting his Nobel Prize in December of 1945, cautioned about the development of bacterial drug resistance, an opinion that was largely ignored [111]. It indeed turned out that the glaring success of penicillin was short lived and the microbes are now coming back with a vengeance. Indications are clear that penicillin, the stalwart of the antibiotic arsenal for the past 60 years, has definitely fallen into hard times. When resistance to methicillin occurred in the 1960s, the large drug companies dedicated their mission to finding even more powerful penicillin variants to overcome persistent infections. With each new analog came a brief period of recovered success, and financial gain, but this strategy soon proved futile. Most of these companies have completely left the antibiotics field in favor of more lucrative drugs for chronic diseases. As a consequence, the stream of FDA-approved antibiotics entering the market has become barely a trickle, and the prospects for turning this around anytime soon do not look very good. Moreover, there are assumptions that the continuing technological development may not fulfill its promise and lead to only escalating drug resistance [112].

Penicillin is just the prototype of many classes of antibiotics that are losing their effectiveness against microbial infection. The steady accumulation of disease-causing microbes in our environment having acquired resistance to penicillin, and the largely ineffective efforts of our public health system to curb the spread of resistance from the hospital out to the community, are alarming facts. It is safe to say that the lessons we have learned over the 60-year history of the β-lactams as clinical agents have been difficult ones. We now recognize the dangers and poor judgment in the careless use and overuse of powerful antibiotics such as penicillins against deadly pathogens. Efforts to curb infectious disease through the reliance on penicillin for six decades, has steadily put pressure on pathogenic microbes to adapt to this new ecosystem. With increasing world population and globalization, it is a sure bet that antibiotic resistance will rapidly accelerate. Antibiotics development has thus hit a conundrum: as bacterial resistance to drugs develops, more powerful drugs are then needed to overcome this resistance. This in turn further pressures microbes to develop even stronger resistance. What is the solution? From the looks of it, we have created quite a mess.

However, throughout the ups and downs of the penicillin era, scientists have not sat idle. We have actually learned a great deal about what works and what does not. We have learned that penicillin first of all is a safe drug, that it is specific for bacteria over mammalian tissue, that it is effective against a wide spectrum of microorganisms, and that it is well tolerated among a highly diverse population living throughout the world. We have also seen how quickly resistance can develop and be transferred from microbe-to-microbe. We are beginning to understand the importance of the bacteria we kill with penicillin, and their role in human health. Most are, in fact, very necessary and killing them brings us to our own demise. We know how and why drug resistance in bacteria takes place, and are beginning to learn how to circumvent the mechanisms that regulate β-lactam resistance. All of this bodes well for ongoing advances in medicine and drug discovery.

Among the most promising opportunities for enhancing efficacy and delivery of β-lactams is with delivery vehicles such as nanoparticles and liposomes. From as far back as the 1940s, emulsified forms of penicillin seemed to offer some advantages for treating microbial infections. Nanoparticles, particular membrane-permeable variants with small diameters, may hold promise, and is an emerging topic for research. Emulsified poly(cyanoacrylate) and poly(acrylate) nanoparticles, in addition to other polymeric matrices, are being explored for applications such as treatment of skin infections and burn wounds, as well as for systemic, non-localized infections. Poly(lactide/glycolide) nanoparticles could be invaluable, as

they are biocompatible, biodegradable nanospheres. As a cautionary note, the application of these drug delivery media in medicine and human health is still largely investigational. Moreover, newly discovered nanotechnologies applied to antibiotics delivery trail far behind that of anticancer agents and therapeutics for other human diseases, and have primarily been focused on biosensors (microbial detection, assay) development. Thus, the investigation of nanoparticle-based antibiotics (nanobiotics) is barely in its infancy. At the present time, there is very little scientific data on the clinical application of nanoparticles as antibiotics and much more investigation must still be carried out to assess toxicity and side effects, as well as efficacy in specific therapeutic applications. Delivery of antibiotics to localities within the human body where infections are difficult to treat, such as the brain, gastrointestinal tract, heart tissue, intracellular, and within bacterial biofilms, is an emerging area of research. Getting the antibiotic to the site of infection, rather than to unaffected tissue where the drug does no good, reduces its exposure to communal bacteria. Targeted drug delivery is an exciting prospect, whether using small recognition ligands appended to the β-lactam directly, or to much larger entities such as nanoparticles, liposomes and proteins that carry β-lactam drugs. Along with this, combination therapy, where several antibiotics of a different class are used simultaneously in a single cocktail, remains a promising strategy that has worked for treating viral infections such as HIV. Synergistic effects are likely, as are broadening the spectrum of antimicrobial activity, and the approach could potentially lower the likelihood for resistance. Delivery vehicles such as liposomes or nanoparticles that can ensure all of the drugs in the cocktail reach the same target microbe together would be ideal, and help avoid the onset of resistance. Acceptance of nanoparticle technology by the biomedical community and the US FDA means that toxicity and human safety in the use of nanomaterials must be adequately addressed.

Beyond the β-lactam delivery vehicles themselves (and drug-conjugated prodrug variants), considerable opportunities for β-lactams exist. The β-lactams do still remain a valuable weapon against infectious diseases, but for them to continue to be, we have to be much more conscientious in how to use them. Many of the antibiotics developed in the past, such as the penicillins, cephalosporins, carbapenems, monobactams and nocardicins, are derived from microbes themselves, which instinctively seem to know when and how to best employ these molecules defensively. We must try to do the same. Just as nature has inspired chemists to devise novel ways to synthesize complex natural products, the same level of creativity must find its way into the design of new antibiotics and drug delivery platforms. The opportunities in this are just staggering. An early example of this ingenuity is in Woodward's introduction of the penems, the sulfur analogs of nature's carbapenems and a ring contracted hybrid of the penicillin and cephalosporin families. More of these innovative constructs await us as synthetic chemists and computational chemists take interest in this field, and as successes begin to accrue. Chemists are now exploring ways to build new drug scaffolds that hit novel cellular targets and processes, and offer alternative modes of action. Examples include, of course, mechanism-based inhibitors of penicillinases and fatty acid biosynthesis (e.g., *N*-thiolated β-lactams). Coupling these to vehicles that can target, time release, peel away and so on, provides the opportunity to deliver the β-lactam drug while protecting it from degradation and elimination.

Other elements of the prior discussion can also be considered for future drug discovery. It is predictable based on past trends that the combined use of β-lactamase inhibitors with β-lactam drugs, per se, will show diminishing returns against β-lactamase-producing strains of bacteria, as inevitably the level of β-lactamase production will ramp up. However, tailoring the β-lactamase inhibitor onto the β-lactam (or its delivery vehicle) provides, potentially, a species-selective or even drug-resistant strain-selective antibacterial prodrug. The employment of β-lactamase-sensitive molecules as biosensors which deliver an antibiotic *only* to drug-resistant microbes is an active area of interest in our own laboratory.

Intracellular bacterial infections represent another challenging, yet largely unexplored, area of investigation [113]. Novel approaches to eradicate hibernating bacteria within human cells are needed, and some β-lactams and penicillin-loaded nanoparticles have been shown to work very effectively against intracellular microbes. This could be potentially a very effective drug delivery strategy, provided that the antibiotic can be introduced into cells at a sufficiently high concentration. Nanoparticles have been shown to introduce antibacterials into macrophages to eradicate intracellular pathogens in the liver and spleen. Much more can potentially be done through further innovation.

Bacterial biofilms are a common cause of recurring infections that are unresponsive to drug therapy, and afflict large numbers of patients who have surgical implants and have survived the initial staph infection. Prophylactic use of β-lactam antibiotics, although not generally encouraged due to drug resistance developing, may in the right setting be useful to avoid the initiation of colonization and biofilm formation. More needs to be done to study this. In regards to eliminating established biofilms, β-lactams may serve of some use, either as antibiotics, or as species-selective sensors of penicillin-binding proteins or β-lactamases as a means to target a second therapeutic (or a nanoparticle) to the site where β-lactamase is in high concentration.

Finally, there are a plethora of new initiatives towards the development of antimicrobially protected biomedical materials and devices, such as sutures, gauzes, wound cleanses, synthetic skins and prosthetics. Each one of these promises a wealth of new opportunities for delivery of β-lactam antibiotics, particularly when coupled to orthogonal tactics such as those discussed above.

Inevitably, complacency comes with success, and this unfortunate curse of human nature will likely continue well into the future and as long as β-lactams are used clinically. By knowing this, secondary measures can and should be implemented to ensure compliance in how our public health community regulates penicillin therapy. The problem of drug resistance is no longer an isolated one that stops at hospital doors or international borders, as microbes and people travel freely together and thus, uniform regulatory measures and surveillance should be adopted internationally. The judicious use of β-lactams in multi-drug therapy and rotational drug therapy (where single drugs are used temporarily but then discontinued before drug resistance develops) is a clear necessity. The success of recovering β-lactams will depend on the implementation of rigid preventative measures within the healthcare community, the monitoring of patients entering hospitals and healthcare centers for pathogenic bacteria, proper training of healthcare workers, as well as enforcement of standardized treatment protocols and routine sterilization procedures. In general, our society has a long way to go yet in making deep inroads into controlling infectious diseases, one which places a high premium on regulated use of antibiotics. This clearly must change. Along with the scientific advances that extend the use of β-lactams, there is a dire need for a change in ideology in how we as a culture deal with infectious diseases and the use of antibiotics. Methods for accurately monitoring and quickly identifying a patients' infection source, as well as to track statistical trends in public health, the typing of the causative microbe, its virulence, and sensitivity to therapy, are necessary measures. As in other disease areas, the increasing push toward personalized medicine – matching a person's disease to a specific treatment regimen – requires availability of effective therapeutics selective for the infectious microbe and tolerable to that patient. Much clearly remains to be done.

The penicillin era is certainly not over, but has turned away from classical medicinal chemistry based drug development toward some exciting new directions. It is our opinion that we are seeing only the very beginning of some very innovative times in antibiotic drug discovery, with β-lactams quite possibly serving again as central players in these developments.

Acknowledgements

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Declaration of interest

E Turos is co-inventor on a US patent application by the University of South Florida for the antibiotic-conjugated polyacrylate nanoparticles. E Turos is currently chief scientific adviser and major shareholder for Nanopharma Technologies, Inc., a University of South Florida spin-out company co-founded by Turos. Nanopharma Technologies, Inc. has licensed the technology from University of South Florida and received a National Science Foundation Small Business Technology Transfer (STTR) Phase II grant to conduct further investigations on this technology, which is being done in collaboration with Turos' laboratory at University of South Florida.

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