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Digest

Antibiotic adjuvants – A strategy to unlock bacterial resistance to antibiotics

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ABSTRACT

Resistance to available antibiotics in pathogenic bacteria is currently a global challenge since the number of strains that are resistant to multiple types of antibiotics has increased dramatically each year and has spread worldwide. To unlock this problem, the use of an '*antibiotic adjuvant*' in combination with an antibiotic is now being exploited. This approach enables us to prolong the lifespan of these life-saving drugs. This digests review provides an overview of the main types of antibiotic adjuvants, the basis of their operation and the remaining issues to be tackled in this field. Particular emphasis is placed on those compounds that are already in clinical development, namely β-lactamase inhibitors.

The discovery of penicillin during the first half of the 20th century and all the antibiotics developed thereafter undoubtedly represents one of the most important achievements in medicine. These drugs have not only saved millions of lives but are also a key tool in the success of common hospital procedures such as general surgery, organ transplantation, dialysis for renal failure and chemotherapy for cancer, for which their capacity to treat secondary infections is crucial. Regrettably, the ability of these drugs to cure infectious diseases is now in serious danger due to the emergence and spread worldwide of strains that are multidrug-resistant to antibiotics.[1] The European Center for Disease Prevention and Control (ECDC) estimates that about 25,000 Europeans die each year as a direct consequence of a multidrug-resistant infection and €1.5 billion is spent in extra patient care costs.[2] According to the U.S. Center for Disease Control & Prevention (CDC), a similar number of deaths also occur in the USA.

The WHO has recently published a global priority pathogens list of antibiotic-resistant bacteria (Table 1).[3] Based on diverse criteria such as mortality, prevalence of resistance, treatability or current pipeline, bacterial pathogens have been ranked as critical, high and medium. This study revealed that the situation is highly critical for healthcare-associated infections caused by the Gram-negative ESKAPE pathogens *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacteriaceae* (including *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter* spp., *Serratia* spp., *Proteus* spp., *Providencia* spp., and *Morganella* spp.). These bacteria are resistant to carbapenems, which are the only remaining therapy that is often considered as antibiotics of last resort.[4] To this list we must add *Mycobacterium tuberculosis*, the causative agent of tuberculosis, which is already considered as a top global health priority. The high and medium categories involve increasingly drug-resistant bacteria that require more monitoring and prevention activities. This priority list of bacterial pathogens is an excellent guide for the prioritization of incentives and funding for R&D aimed at discovering new and effective antibacterial therapies, which is undoubtedly necessary.[5]

A recent study has shown that resistance to antibiotics is a natural phenomenon that predates the golden age of antibiotic therapy due to the intrinsic evolutionary character and adaptability of bacteria.[6] Nonetheless, human behavior has undoubtedly been decisive in reaching bacterial resistance to the current alarming levels. One of the key factors is the abuse and misuse of these drugs over the years in humans, animals and plants, including for the treatment of non-bacterial diseases. As shown recently, this behavior has favored the appearance of tolerance and this, in turn, facilitates the emergence of resistance.[7] Another critical point that is limiting our capacity to deal with multidrug resistant bacteria is the flagging investment in R&D on novel antibiotics by the large pharmaceutical companies since the 1960s. This is mainly due to: (a) the huge economic cost of bringing a drug to the market;

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Table 1
WHO priority bacterial pathogens list for research and development of new antibiotics.[a]

Priority	Bacterial Pathogen
Critical	*Acinetobacter baumannii*, carbapenem-resistant *Pseudomonas aeruginosa*, carbapenem-resistant *Enterobacteriaceae*,[b] carbapenem-resistant, 3rd generation cephalosporin-resistant *Mycobacteria*, including *Mycobacterium tuberculosis*
High	*Enterococcus faecium*, vancomycin-resistant *Staphylococcus aureus*, methicillin-resistant, vancomycin intermediate and resistant *Helicobacter pylori*, clarithromycin-resistant *Campylobacter*, fluoroquinolone-resistant *Salmonella* spp., fluoroquinolone-resistant *Neisseria gonorrhoeae*, 3rd generation cephalosporin-resistant, fluoroquinolone-resistant
Medium	*Streptococcus pneumoniae*, penicillin-non-susceptible *Haemophilus influenza*, ampicillin-resistant *Shigella* spp., fluoroquinolone-resistant

[a] Ref. 3.
[b] It includes *Klebsiella pneumonia*, *Escherichia coli*, *Enterobacter* spp., *Serratia* spp., *Proteus* spp., and *Providencia* spp., *Morganella* spp.

(b) the low profit margin for non-chronic diseases of this type compared with continuing ones such as cancer or mental diseases and (c) the belief that the great therapeutic arsenal available was sufficient to deal with this issue.[8] As a consequence, the clinical pipeline for antibiotics is almost empty compared to more than 500 chronic-disease drugs for which resistance is not an issue.

It is also relevant that most of the antibiotics in clinical use target a small number and the same type of key functions for bacterial survival and resistance to them is widespread and well known (Table 2).[9]

Specifically, the available antibiotics target: (1) cell-wall biosynthesis; (2) protein synthesis (subunit 30*S* or 50*S* of ribosome); (3) DNA replication and repair (RNA polymerase, DNA gyrase); (4) folic acid metabolism; and (5) membrane structure. An analysis of the antibiotics currently in clinical development (about 40 small molecules) revealed that unfortunately this trend has not changed significantly (Fig. S1).[10] The Pew Charitable Trust highlighted that nearly all of the antibiotics approved in the last 30 years are modifications of earlier classes to make them more efficient and robust against resistant bacteria. Specifically, only two of them are compounds with a new mechanism of action (Fig. 1): (a) Brilacidin (PMX-30063, Phase 2, Cellceutix Corp.) is a synthetic mimetic of host defense protein, which is the first line of defense against microbial infection in many species; and (b) ACHN-975 (Phase 1, Achaogen Inc.), which is an inhibitor of LpxC – an enzyme involved in the Lipid A biosynthesis.

Table 2
Bacterial targets of antibiotics in clinical use.

Target	Type of Antibiotic[a]
Cell-wall biosynthesis	Penicillins, cephalosporins, carbapenems, monobactams, cycloserine, fosfomycin, glycopeptides, lipoglycopeptides
Protein synthesis	Aminoglycosides, tetracyclines (*Subunit 30S*) Oxazolidinones, macrolides, thiopeptides, chloramphenicol, fusidic acid, clindamycin (*Subunit 50S*)
DNA replication and repair	Rifamycins, ansamycins, actinomycins, tiacumycins (*RNA polymerase*) Fluoroquinolones, aminocoumarins (*DNA gyrase*)
Folic acid metabolism	Sulfonamides, trimethoprim
Membrane structure	Lipopeptides, polymyxins

[a] The target is included in brackets.

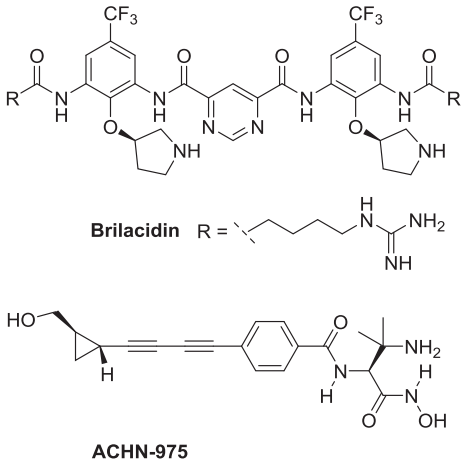

Fig. 1. Antibiotics in clinical trials that have a new mechanism of action.

Taken together, it seems clear that it is crucial to search not only for more effective anti-infective drugs but also to develop novel chemical entities with new mechanisms of action. A great deal of effort is currently being devoted, particularly in academia, to investigate the potential of unexploited essential processes in bacteria and to develop novel scaffolds that target them, as well as to study the biochemical basis of these targets in detail. The increasing availability of bacterial genome sequences in general, and the identification of the essential genes for bacterial survival in particular, have contributed greatly to the progress made in this area of research. However, the development of antibiotics that act on novel targets is a very challenging and expensive task, as evidenced by the fact that there are only two compounds in clinical trials that meet this premise. A good example is CARB-X (Combating Antibiotic Resistant Bacteria Biopharmaceutical Accelerator), the world's largest public-private partnership, which was recently created and is devoted antibacterial preclinical R&D.[11] Funded by BARDA and the UK's Wellcome Trust, and supported by NIAID, CARB-X will spend $450 million from 2017–2021 to bring innovative treatments toward clinical trials. In contrast, greater success has been achieved with those approaches that minimize the emergence and impact of resistance to antibiotics by the use of an '*antibiotic adjuvant*' in combination with an antibiotic. This booming and successful strategy will be the focus of this digest review. An overview of the recent efforts carried out on such combined therapies to face the challenge of multidrug resistance to antibiotics is reviewed.

Antibiotic Adjuvants: These compounds are also named '*resistance breakers*' or '*antibiotic potentiators*'[12–21] and they have little or no antibiotic activity but co-administered with the antibiotic they either (i) block the main bacterial resistance mechanisms or (ii) enhance the antimicrobial action of the drug. From the drug discovery point of view, this combined drug therapy has the advantage that it is not necessary to expend effort in the challenging and expensive identification of new targets that are essential for bacterial survival.

There are four main mechanisms of antibiotic resistance (Fig. 2)[22]:

(1) Enzymatic inactivation of the antibiotic – an existing cellular enzyme is modified to react with the antibiotic in such a way that it no longer affects the bacteria. One of the most relevant examples are β-lactamase enzymes, which hydrolyze the most widely used antibiotics, i.e., β-lactams (penicillins and cephalosporins), and represent the most prevalent cause of antibiotic resistance in Gram-negative bacteria.

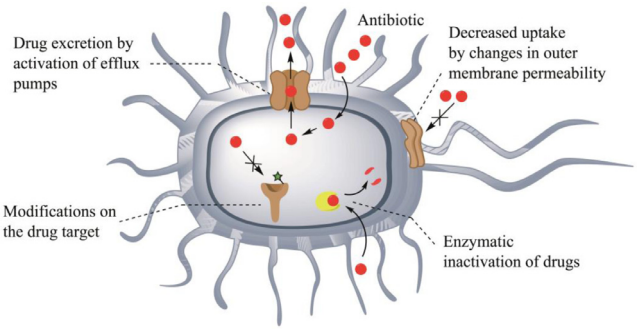

Fig. 2. Schematic representation of the main bacterial mechanisms of antibiotic resistance.

(2) Drug excretion by activation of efflux pumps – these proteins, which are able to eliminate a wide variety of compounds from the periplasm to the outside cell, are activated by the bacteria to eliminate the antibiotic. This is a particularly important resistance mechanism in *P. aeruginosa* and *Acinetobacter* spp.
(3) Decreased uptake by changes in the outer membrane permeability – these variations hinder the effective entrance of the antibiotic.
(4) Modifications on the drug target – these changes decrease or destroy the binding efficiency of the antibiotic and therefore limit its potency.

To block the aforementioned mechanisms of antibiotic resistance, three main types of antibiotic adjuvants have been developed: (a) β-lactamase inhibitors; (b) efflux pump inhibitors; and (c) outer membrane permeabilizers. In addition, to enhance the antimicrobial action of the drug, a great deal of research has recently been devoted to exploring the potential of targeting *bacterial pathogenicity*, i.e., the capacity of the bacterium to cause an infection.[23] The attenuation of bacterial virulence will make the bacterium less able to establish successful infection and, in consequence, it would be cleared by the host immune response or the antibiotic. Anti-virulence drugs have not yet reached clinical trials, but it seems to be a promising strategy since these compounds would create an *in vivo* scenario similar to that achieved by vaccination with a live attenuated strain. Excellent reviews of the compounds identified from natural sources or synthetically developed to target bacterial virulence have been published.[24] An overview of the antibiotic adjuvant strategies is provided below.

β-Lactamase Inhibitors. They are the most successful and clinically used antibiotic adjuvants to overcome resistance to β-lactam antibiotics, which despite 70 years of clinical use still remain at the forefront of antibiotic chemotherapy.[25] These inhibitors protect antibiotics from a very efficient bacterial inactivation mechanism involving β-lactamases (EC 3.5.2.6). These enzymes hydrolyze the β-lactam core of β-lactams in an acylation-deacylation-based process. Based on protein sequence similarities, four major β-lactamase classes (A, B, C and D) are known. The class A, C and D enzymes are serine-β-lactamases whereas class B enzymes are metallo-β-lactamases that use one or two Zn^{2+} ions coordinated to histidine, cysteine and aspartate residues to catalyze the hydrolysis of the β-lactam bond. Among them, carbapenem-hydrolyzing class D β-lactamases (CHDLs), which are also known as 'oxacillinases (OXAs)', are the most rapidly growing and diverse group of β-lactamases with over 500 reported enzymes.[26] They are found among the most clinically challenging species including *A. baumannii*, *P. aeruginosa*, *E. coli*, and *Proteus mirabilis* and they are able to hydrolyze penicillins, extended spectrum cephalosporins and aztreonam. It is therefore not surprising that significant effort is currently been devoted to the development of efficient inhibitors against the most important CHDLs in clinical settings, namely OXA-23, OXA-24/40 and OXA-48. From the drug design point of view, the CHDL enzymes are very challenging due to the uncommon structure of their active site in comparison with the other β-lactamases (A–C). Thus, it has been proposed that the ability of these enzymes to hydrolyze carbapenems is provided by a tunnel-like entrance to the active site formed by the side chains of a tyrosine or phenylalanine and a methionine (Fig. 3). In general, this tunnel-like structure of CDLDs enzymes forms a hydrophobic barrier that controls access to the active site to only certain substrates and it remains mainly unchanged after ligand binding.[27] In practice, this structure of the active site results in a more limited efficiency of the available β-lactamase inhibitors in clinical use, i.e., clavulanic acid (**1**), sulbactam (**2**) and tazobactam (**3**) (Fig. 4A). These compounds, which are mechanism-based covalent inhibitors that make a stable adduct through the catalytic serine, are clinically ineffective against class C and D β-lactamases. Three main types of inhibitors have been developed recently to overcome this limitation and these are summarized below.

Penicillin-based sulfones – In the early 1990s and after the discovery of clavulanic acid, the first β-lactamase inhibitor to be used in the 1980s in combination with penicillins, such as amoxicillin, which was isolated from the bacteria *Streptomyces clavuligerus* by Beecham Group (now part of GSK) in 1976,[28] the first penicillin sulfones, sulbactam (**2**) and tazobactam (**3**), were developed. In contrast to clavulanic acid, compounds **2** and **3** undergo a ring opening after nucleophilic attack by the catalytic serine due to the formation of a sulfinate group, which is absent from the β-lactam antibiotics (sulfide). The generation of this good leaving group, in combination with the extra electrostatic interactions with relevant residues of the active site, is key for the efficiency of these irreversible inhibitiors. In 1986, researchers from Pfizer found that the incorporation a (2-pyridyl)methylene group at C6 of the sulbactam, compound **4a**, increases its efficacy against β-lactamases from *S. aureus* and *E. coli* (micromolar) due to the formation of an indolizine adduct **5**, which is resistant to hydrolysis (deacylation) (Fig. 4B).[29] Related with the latter, in 1989 Coleman et al.[30] developed a very potent penem-based inhibitor, namely **BRL 42715**, that at concentrations of 0.25 μg/mL or less enhanced the activity of amoxicillin against many β-lactamases. Despite the very high efficiency of this compound its instability was an issue. It was subsequently shown that the inhibitory efficacy of **4a** increases dramatically upon incorporation of a catechol moiety, compound LN-1-255 (**4b**), that also facilitates internalization through the outer membrane via bacterial iron uptake pathways.[31,32] The use of such a Trojan horse strategy that involves the incorporation of iron chelating moieties (siderophores) in the compound has increased dramatically in recent years in antibiotic drug discovery.[33] This approach is currently being exploited in antibiotics in clinical trials and these include monobactams MC-1 and BAL-30072 and cephalosporin S-649266. The inspiration for the use of such chelating groups comes from nature, as they are present in diverse natural products produced by bacteria (enterobactin, ferrichrome, pyochelin, pyoverdine, etc.). LN-1-255 proved to be very efficient *in vitro* against the most worrisome and clinically relevant CHDLs in *A. baumannii* and it had an efficacy of inhibition that was 10–1000-fold better than tazobactam or avibactam and it also lowered MIC values from 32 to 2 μg/mL.[34] LN-1-255 represents a potential new therapeutic option in combination with carbapenems or cephalosporins against resistant *A. baumannii* strains. Future preclinical studies on infection animal models are expected to show the potential of this promising and very efficient inhibitor.

Diazabicyclooctanes – Other β-lactamase inhibitors that have already shown their potential as antibiotic adjuvants are diazabicyclooctanes (DBOs) (Fig. 4C). These compounds, which were first

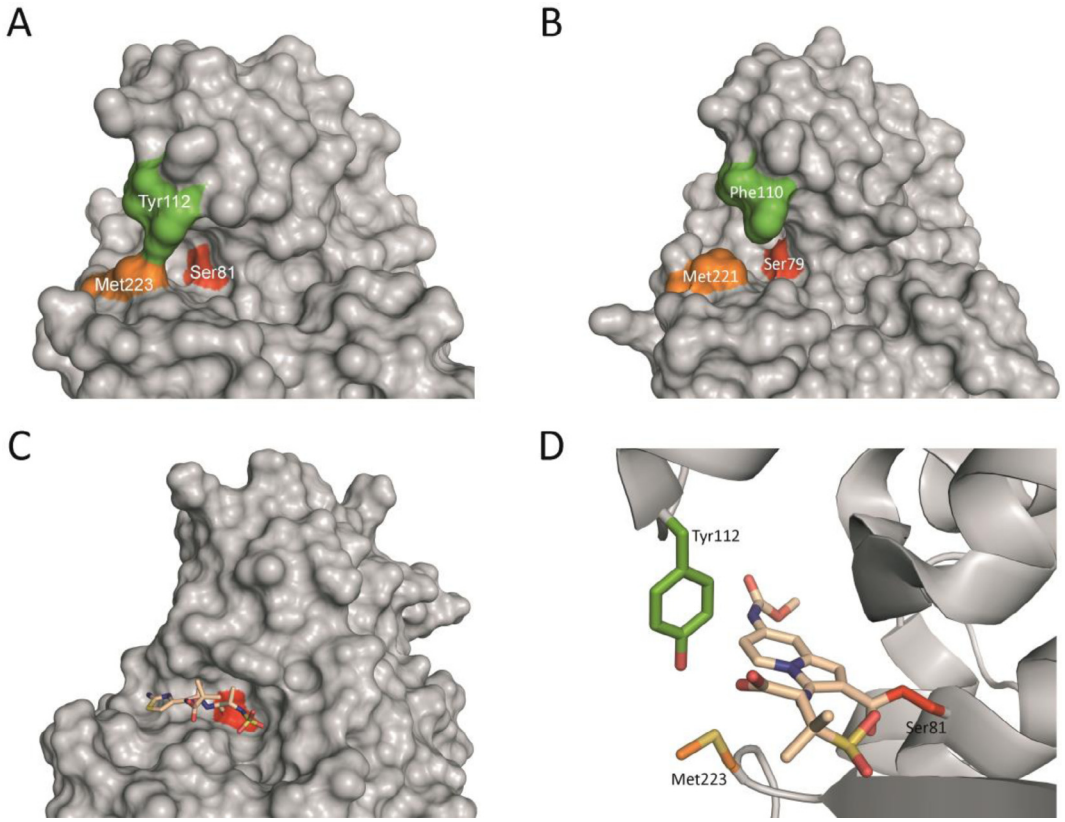

Fig. 3. Overall structures of class A and D β-lactamase enzymes: (A) OXA-24 from *A. baumannii* (PDB code 3G4P,[32] 1.1 Å). (B) OXA-23 from *A. baumannii* (PDB code 4K0X,[36] 1.31 Å). (C) CTX-M-97/Aztreonam adduct from *E. coli* (PDB code 5G18,[37] 1.1 Å). (D) OXA-24/penicillin-based sulfone adduct from *A. baumannii* (PDB code 3FV7,[32] 2.0 Å). The position of the tunnel-like entrance to the active site in (A) and (B) involving Tyr112 and Phe110 (green) and Met223 and Met221 (orange) is highlighted. The position of the catalytic serine is shown in red. Note how the catalytic serine is well protected from the solvent environment for class D enzymes (A,B).

investigated as β-lactam mimics in the mid-1990s by Hoechst Marion Roussel (now part of Sanofi-Aventis), were found to be efficient β-lactamase inhibitors. DBOs react with the enzyme by formation of a carbamoyl adduct that does not undergo further ring-opening transformations. This distinct reactivity of DBOs allows the regeneration of intact inhibitor without hydrolysis during the enzymatic deacylation process.[35]

Avibactam (**6a**), which was the pioneer DBO in clinical use, was approved in late 2014 by the FDA in combination with ceftazidime for the treatment of complicated intra-abdominal and complicated urinary tract infections.[38] Avibactam (**6a**) displays excellent inhibitory activity against most class A and C enzymes, such as TEM-1, KPC-2 and P99, and also proved to inhibit penicillin-binding proteins (PBPs).[39] However, this compound showed a more variable efficiency toward CHDLs and in some of them, mainly OXA-23 and OXA-24, which are mainly responsible for carbapenem-resistance in *A. baumannii*, they were not efficient.[34b] Durand-Réville et al.[40] have shown recently that the activity of avibactam (**6a**) against the latter enzymes can be improved by the incorporation of a double bond and a methyl group in the six membered ring, compound ETX2514 (Fig. S2). It has been suggested that the poorer access to the active site of some of these OXA enzymes is responsible for this reduced efficiency.[41] Structural and kinetic studies revealed a key role of serine and lysine residues in the deacylation process that regenerates the inhibitor and highlighted the structural differences between β-lactamase enzymes to explain its reactivity (Fig. S3).[42] Other combination therapies involving avibactam (**6a**) and further developed DBO compounds, such as relebactam (**6b**) and zidebactam (**6d**), are in clinical trials (Table 3). Moreover, OP0595 (**6c**), a recently developed DBO compound that is in Phase 1 clinical trials, in addition to being a class A and C β-lactamase inhibitor was found to be an inhibitor of PBP2 and several *Enterobacteriaceae* and also an enhancer of β-lactam antibiotics due to binding to other PBPs besides PBP2.[43]

Boronic acids as transition state analogs – These compounds were developed after the discovery that serine proteases can be inhibited by boronic acids.[44] Kiener and Waley[45] applied this idea to the class A β-lactamase from *B. cereus* and showed that diverse boronic acid derivatives were inhibitors of the enzyme in the mM range (Fig. 5). The inhibition is due to the formation of a tetrahedral intermediate with the catalytic serine that mimics the transition state in the hydrolytic reaction catalyzed by β-lactamase (Fig. 5A).[46] The latter finding – in combination with further modification of the initial scaffolds mainly by the incorporation of side chains similar to those observed in the natural penicillins [(thiophen-2-yl)acetamido group] and the introduction of extra binding interactions in the pocket close to the catalytic serine (compounds **7**–**11**) – led to a decrease in the inhibition potency against clinically relevant class A and C β-lactamase enzyme to the nanomolar range, e.g., compound **11** (K_i = 1 nM) (Fig. 5B).[47] The resolution of diverse crystal structures of the AmpC β-lactamase/adduct complexes provided a detailed knowledge of the basis of the high affinity of these transition state mimetics that explored two pockets close to the catalytic serine (Fig. S4).[47b] Moreover, fragment-guided design involving the replacement of the amide moiety by diverse sulfonamides provided K_i values in the subnanomolar range, e.g., compounds **12**–**15**.[48] *In vivo* studies in mice infected with β-lactamases expressing *E. coli* showed that the combination of cefotaxime and compound **15** is able to cure the infection in 65% yield.

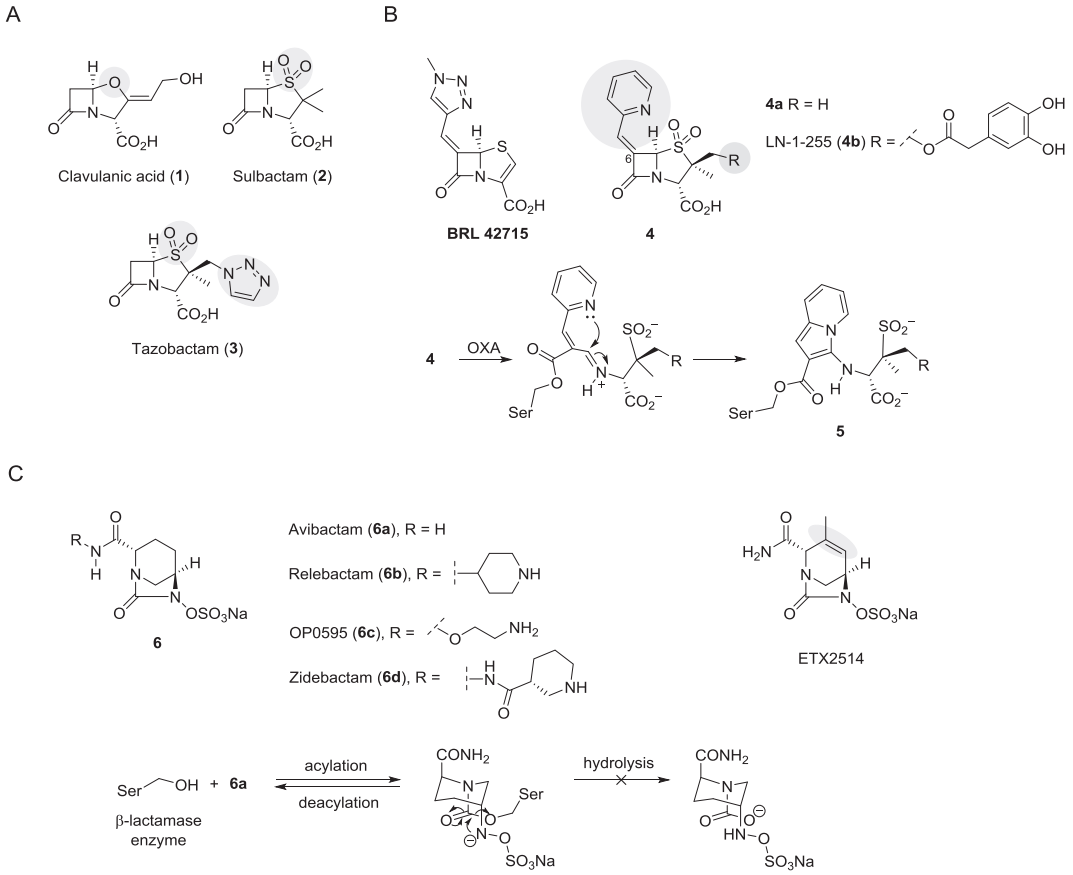

Fig. 4. Relevant β-lactamase inhibitors: (A) compounds in clinical use. (B) 6-Alkylidene penicillin sulfones with excellent *in vitro* efficacy. (C) Diazabicyclooctanes in clinical trials and their mechanism of action.

Table 3
Combination drug therapies (antibiotic + β-lactamase inhibitor) in clinical trials.

Name	Antibiotic	β-Lactamase inhibitor	Phase, Company	Possible application
WCK5222	Cefepime	Zidebactam	Phase 1, Wockhardt Ltd	Complicated urinary tract infections, hospital-acquired-/ventilator-associated bacterial pneumonia
ATM-AVI	Aztreonam	Avibactam	Phase 2, AstraZeneca	Complicated intra-abdominal infections
CXL	Ceftaroline fosamil	Avibactam	Phase 2, Pfizer Inc./ Allergan PLC	Multi-resistant bacterial infections
MK-7655[a]	Imipenem	Relebactam	Phase 3, Merck Sharp & Dohme Corp.	Complicated urinary tract infections, complicated intra-abdominal infections, hospital-acquired-/ventilator-associated bacterial pneumonia, acute pyelonephritis
Carbavance	Biapenem	RPX7009	Phase 3, Rempex Pharmaceuticals	Complicated urinary tract infections, complicated intra-abdominal infections, hospital-acquired-/ventilator-associated bacterial pneumonia, febrile neutropenia, bacteremia, acute pyelonephritis

[a] It also includes Cilastatin, an inhibitor of the human dehydropeptidase enzyme, which is found in the kidney and is responsible for degrading Imipenem.

Subsequent evaluation of diverse cyclic boronate esters designed to improve the selectivity towards β-lactamases *vs* other serine hydrolases that transform linear substrates allowed the identification of RPX7009.[49] This compound was able to restore the activity of carbapenems against *K. pneumoniae* carbapenemase (KPC). A combination with biapenem at 4 μg/mL was very efficient, with MIC_{50} values of 0.12 μg/mL against KPC-producing *Enterobacteriaceae* that coexpresses between one and four additional β-lactamases (including class A, CTX-M and non-carbapenemase OXA) or hyperexpressed chromosomal AmpC enzymes.[50] However, this efficiency was not seen with *Enterobacteriaceae* expressing other β-lactamases, such as OXA-48. This combination drug therapy, named as Carbavance, is currently under Phase 3 clinical trials.

Efflux Pump Inhibitors (EPIs). In 1980, McMurry et al.[51] first demonstrated that active export of tetracycline is a common ingredient of the bacterial resistance mechanism to this drug. It was later shown that this is in fact a widely extended phenomenon for a wide variety of antibiotics that decreases their efficiency by between 1- and 64-fold.[52–59]

This finding triggered significant effort to discover inhibitors of active efflux pumps as an attractive strategy for restoring the activity of existing antibiotics. Further studies revealed that this mechanism of resistance is non-specific and, as a consequence, the identification and development of efficient EPIs proved to be very challenging and this has hampered their discovery. The most common antibiotic-efflux systems in pathogenic bacteria, such as *P. aeruginosa,* are the Major Facilitator Superfamily (MFS) and Resistance/Nodulation/Division Superfamily (RND). A wide variety of compounds, either synthetic or from natural sources, have been reported and have recently been reviewed.[60] These compounds

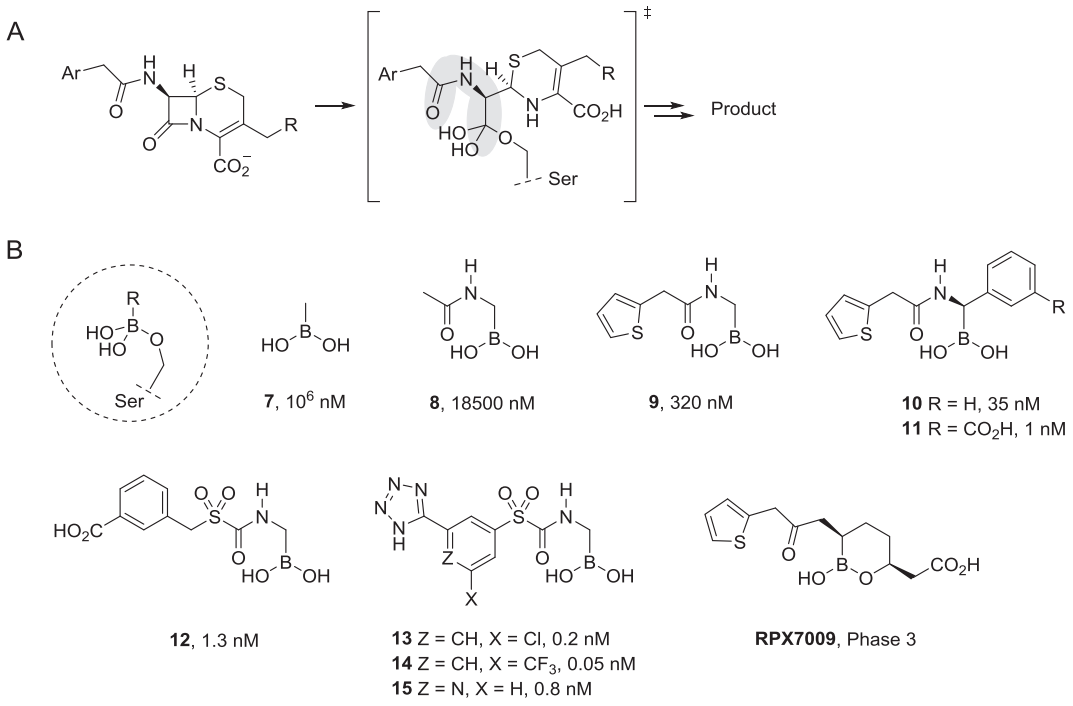

Fig. 5. Boronic acid β-lactamase inhibitors. (A) General structure of the transition state of the reaction catalyzed by β-lactamase enzymes. (B) Selection of the most relevant boronic acid β-lactamase inhibitors. K_i values against AmpC β-lactamase enzyme are also indicated.

are tetracyclines, piperidines, aminoglycosides, quinolines, pyridopyrimidines and arylpiperazines, among others.

Outer Membrane Permeabilizers. The limited efficiency of some antibiotics for the treatment of infections caused by Gram-negative bacteria is largely due to the particularly complex structure of their membrane. Specifically, the membrane is well designed with an extra layer (the outer membrane) that is mainly composed of polyanionic lipopolysaccharides, which limits the entrance of small molecules such as antibiotics. The use of permeabilizers proved to be a good approach to improve the antibiotic uptake. These compounds are usually cationic and amphiphilic or are chelators that destabilize the outer layer of the outer membrane by either interaction with the polyanionic lipopolysaccharides or by capture of outer layer cations, respectively. As a consequence, this region of the outer membrane becomes more permeable, thus facilitating the drug uptake. Examples of outer membrane permeabilizers are polymyxins, such as colistin, aminoglycosides, cationic peptides, cationic cholic acid derivatives or polyamines.[61] It is worth highlighting that phenylalanine-arginine β-naphthylamide (**PAβN**, also called MC-207,110),[62] which is one of the most studied EPIs and has proved to be efficient in reducing the resistance to fluoroquinolone antibiotics in *P. aeruginosa*, has recently been shown to permeabilize bacterial membranes. In particular, **PAβN** proved to have similar activity to Polymyxin B nonapeptide in *E. coli*[63] and enhanced the efficacy of β-lactams against wild type and AmpC-overexpressing strains of *P. aeruginosa*.[64] It is therefore possible that **PaβN** might have a dual antibiotic adjuvant action.

Outlook: Among the strategies that are currently being explored to unlock the worldwide emergence and spread of multidrug resistant bacteria, the use of an '*antibiotic adjuvant*' in combination with an antibiotic has proven to be very efficient with several combinations currently in clinical studies. This approach has two main advantages: (i) the lifespan of the available antibiotic arsenal can be prolonged, and (ii) the pressure on the challenging development of novel chemical entities that disable unexplored bacterial targets can be relaxed. The principal disadvantages of their use are: (i) as with other combined therapies, the risk of adverse effects due to potential drug-drug interactions, and (ii) more complex studies are required to establish effective codosing regimes since compatible pharmacokinetic and pharmacodynamic properties between the antibiotic and adjuvant antibiotic are required.

Blocking the enzymatic inactivation of β-lactam antibiotics by β-lactamase inhibitors is without doubt the most validated approach, with numerous compounds, such as clavulanic acid (**1**), sulbactam (**2**) and tazobactam (**3**), already in clinical use and many others in clinical development. This success has relied on crucial detailed studies on the mechanism of action and the extensive structural and biochemical knowledge of the β-lactamase enzymes. The lack of such knowledge for the other types of antibiotic adjuvants summarized here has hampered their expansion. Efforts devoted to this aspect would help to enhance the efficacy and improve toxicity profiles of the outer membrane permeabilizers as well as EPIs.

In spite of the advances in the use of β-lactamase inhibitors, there is an urgent need to develop effective inhibitors of class B β-lactamases (metallo-β-lactamases), for which at present there are no inhibitors available, and their dissemination among relevant Gram-negative bacteria, such as Enterobacteriaceae, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli* and *Acinetobacter baumannii* is growing dramatically.[65] Significant effort must also be devoted to the inhibition of CDLD enzymes, particularly those with a more closed active site, such as OXA-24/40, which are commonly present in the most clinically challenging species that have been classified as 'bacterial pathogens of critical priority' by the WHO.

Acknowledgments

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A. Supplementary data

The list of the antibiotics currently in clinical development classified by target (Fig. S1), 3D models for sulbactam, tazobactam, BRL 42715, LN-1-255, avibactam and RPX7009 and detailed view of several enzyme/inhibitors adducts (Figs. S2−S4). Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.08.027. These data include MOL files and InChiKeys of the most important compounds described in this article.

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