Drug Resistance Updates 36 (2018) 13–29

Contents lists available at ScienceDirect

Drug Resistance Updates

journal homepage: www.elsevier.com/locate/drup

Invited review

An update on β-lactamase inhibitor discovery and development

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ARTICLE INFO

Keywords:
Beta-lactamase
Inhibitor
Crystal structure
Antibiotic resistance
Drug discovery

ABSTRACT

Antibiotic resistance, and the emergence of pan-resistant clinical isolates, seriously threatens our capability to treat bacterial diseases, including potentially deadly hospital-acquired infections. This growing issue certainly requires multiple adequate responses, including the improvement of both diagnosis methods and use of antibacterial agents, and obviously the development of novel antibacterial drugs, especially active against Gram-negative pathogens, which represent an urgent medical need.

Considering the clinical relevance of both β-lactam antibiotics and β-lactamase-mediated resistance, the discovery and development of combinations including a β-lactamase inhibitor seems to be particularly attractive, despite being extremely challenging due to the enormous diversity, both structurally and mechanistically, of the potential β-lactamase targets. This review will cover the evolution of currently available β-lactamase inhibitors along with the most recent research leading to new β-lactamase inhibitors of potential clinical interest or already in the stage of clinical development.

1. Introduction – the relevance of β-lactamases as drug targets

Antibacterial resistance has now reached alarming levels, with major bacterial pathogens (including Gram-negative organisms such as *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*) quickly evolving towards pandrug resistance phenotypes (Monaco et al., 2014; Rossolini et al., 2014). Without an adequate response to this medical issue, the burden and mortality associated to infectious diseases, and especially hospital-acquired bacterial infections, is consequently expected to significantly increase in the near future (O'Niel, 2014; World Health Organization, 2014).

β-lactam antibiotics, the first natural antibacterial compounds to be successfully developed, and subsequently modified, by the pharmaceutical industry, still represent an outstanding class of antibiotics, thanks to both their excellent antibacterial activity and selectivity, accounting for over 65% of injectable antibiotics in the clinical setting in the U.S.A. (Bush and Bradford, 2016). However, and like any other antibacterial drug used in the clinical setting so far, their use is soon or later followed by the emergence of resistant strains which, following the acquisition of resistance determinants, can rapidly spread on a global scale (the rapid and global diffusion of NDM-1-producing *K. pneumoniae* isolates is a striking example) (Dortet et al., 2014; Khan et al., 2017).

Mechanisms of resistance to β-lactams include the production of efflux pumps, the modification or reduced production of outer membrane porins (in Gram-negative bacteria), alterations of Penicillin-Binding Proteins (the molecular target of β-lactams) and the production of an enzyme able to inactivate the antibiotic (a β-lactamase) (Frère et al., 1991).

β-lactamase production represents the most relevant mechanism of resistance in Gram-negative pathogens. For that reason, the pharmaceutical industry successfully employed two strategies to overcome β-lactamase-mediated resistance to β-lactams: (a) the optimization of β-lactamase-stable antibiotics (such as the expanded-spectrum cephalosporins and carbapenems that are resistant to hydrolysis by narrow-spectrum β-lactamases or extended-spectrum β-lactamases, respectively) and (b) the development of selective β-lactamase inhibitors (BLIs) to be co-administered with a β-lactam antibiotic. In that perspective, the discovery of a *Streptomyces clavuligerus* secondary metabolite, clavulanic acid (Scheme 1, **a**), represented a significant milestone in the field of antibacterial discovery. This β-lactam molecule is able to specifically inhibit most of the β-lactamases circulating in the 80's, while showing a modest (if any) antibacterial activity, and allowed for the development of the first β-lactam-β-lactamase inhibitor combination, Augmentin (amoxycillin/clavulanate). This drug encountered a huge commercial success and was followed by the introduction of other combinations with tazobactam (Scheme 1, **b**) and sulbactam (Scheme 1, c).

The fast evolution of β-lactamases, with an exponentially-increasing number of variants showing an impressive diversification of functional

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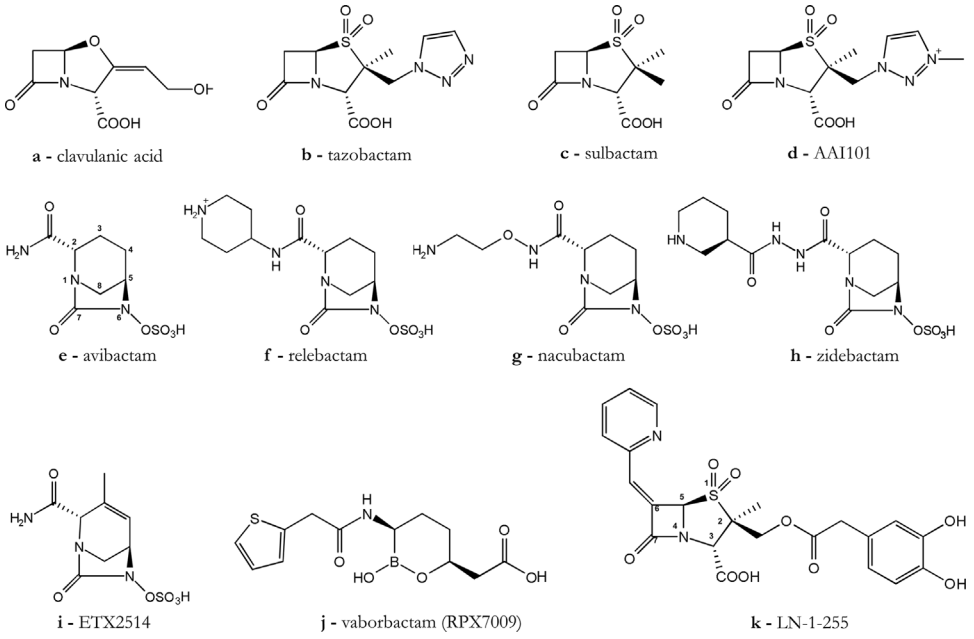
https://doi.org/10.1016/j.drup.2017.11.002
Received 20 June 2017; Received in revised form 1 November 2017; Accepted 3 November 2017



Scheme 1. Chemical structures of β-lactamase inhibitors, showing their expanding chemical diversity. Besides the β-lactam compounds (clavulanic acid, tazobactam, sulbactam and AAI101, **a–d**), new chemical entities showing an expanded spectrum of inhibition successfully entered the stage of clinical development: diazabicyclooctanone compounds (avibactam, relebactam, nacubactam, zidebactam and ETX2514, **e–i**) and boronic acids (RPX7009, **j**). Chemical structure of the alkylidene penam sulfone LN-1-255 (**k**), showing the presence of unusual chemical substituents at positions 2 and 6.

properties, including the ability to hydrolyze the carbapenems and/or being insensitive to available β-lactam-based β-lactamase inhibitors surely represent an important factor in the evolution of relevant Gram-negative pathogens towards multi-drug resistance (MDR) and extensively-drug resistance (XDR) phenotypes (Bush, 2013). For that reason, colistin, a polymyxin antibiotic is progressively replacing carbapenems as last-resort agents to treat complicated infections caused by carbapenem-resistant XDR pathogens (including carbapenem-resistant isolates of *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*), despite presenting some potential safety issues and requiring a very tightly controlled administration regimen (Biswas et al., 2012).

The rise of antibiotic resistance in the last two decades and the growing need for new antibacterial drugs contributed to a significant regain of interest towards β-lactam/β-lactamase inhibitor combinations. Still considered among the most reliable strategies to deliver antibacterial drugs able to address the development of resistance, a significant number of new combinations entered the stage of clinical development since 2010 (Table 1). One of the first successes deriving from these efforts led to the approval, in March 2015, of the first non-β-lactam β-lactamase inhibitor, avibactam (Scheme 1, **e**), available in combination with ceftazidime (4:1 ratio) for the treatment of complicated urinary tract infections. Following avibactam, the first member of the diazabicyclooctonane class of β-lactamase inhibitors, other structurally-related β-lactamase inhibitors also reached the stage of clinical development, including relebactam (MK-7655) (Scheme 1, **f**), developed by Merck in combination with imipenem-cilastatin, nacubactam (FPI-1459, RG6080, OP0595) (Scheme 1, **g**), zidebactam (currenlty developed in combination with cefepime) (Scheme 1, **h**) and ETX2514 (Scheme 1, **i**). The two latter compounds interestingly show significant inhibition of both β-lactamases and penicillin-binding proteins (Morinaka et al., 2015; Shapiro et al., 2016). Finally, an additional class of non-β-lactam β-lactamase inhibitors, boronic acids (Scheme 1, **j**), successfully reached the final stages of clinical development (meropenem-vaborbactam [RPX7009], also known as Carbavance or Vabomere) and was approved in 2017.

In this review, the major advances in β-lactamase inhibitor discovery and development will be presented, with a focus on the structural basis for inhibition of clinically relevant β-lactamases and the emerging mechanisms of resistance to available next-generation β-lactam/β-lactamase inhibitor combinations.

Table 1
β-lactam-β-lactamase inhibitor combinations that reached the stage of clinical development since 2010.

Combination	Status
Ceftolozane-tazobactam (2:1)	Approved in 2014 in the U.S.A. and in 2015 in Europe
Ceftazidime-avibactam (4:1)	Approved in 2015 in the U.S.A. and in 2016 in Europe
Ceftaroline-avibactam	Various phase 1 trials completed 2011–2017
Aztreonam-avibactam	Phase 1 studies initiated in 2012 (completed in 2016) and 2015
Meropenem-Vaborbactam	Approved in 2017 in the U.S.A.
Imipenem-cliastatin-relebactam (2:2:1)	Phase 3 initiated in 2015
Nacubactam[a]	Phase 1 trials completed in 2014 and in 2017
Cefepime-zidebactam	Phase 2 completed in 2016
Cefepime-AAI101	Phase 2 initiated in 2017
Sulbactam-ETX2514	Phase 1 trials completed in 2017
VNRX – 5133[a]	Phase 1 initiated in 2017

[a] β-lactamase inhibitor, partner β-lactam currently unspecified.

2. "Classical" β-lactamase inhibitors – oxapenam and penam sulfones – and their evolution

Clavulanate, tazobactam and sulbactam, the three β-lactam BLIs commercially available, behave as "mechanism-based" β-lactamase inactivators. They all irreversibly form a covalent adduct with the catalytic serine of serine-β-lactamases, which further evolves via slow hydrolysis or fragmentation of the inhibitor molecule. Interestingly, the fragmentation of the inhibitor can lead to the formation of inactivated β-lactamase adduct whose half-life is even longer than the initially formed acyl-enzyme (Kuzin et al., 2001; Matagne et al., 1993). The