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The latest advances in β-lactam/β-lactamase inhibitor combinations for the treatment of Gram-negative bacterial infections

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Abstract

Introduction: Antimicrobial resistance in Gram-negative pathogens is a significant threat to global health. β-Lactams (BL) are one of the safest and most-prescribed classes of antibiotics on the market today. The acquisition of β-lactamases, especially those which hydrolyze carbapenems, is eroding the efficacy of BLs for the treatment of serious infections. During the past decade, significant advances were made in the development of novel BL-β-lactamase inhibitor (BLI) combinations to target β-lactamasemediated resistant Gram-negatives.

Areas covered: The latest progress in 20 different approved, developing, and preclinical BL-BLI combinations to target serine β-lactamases produced by Gram-negatives are reviewed based on primary literature, conference abstracts (when available), and US clinical trial searches within the last 5 years. The majority of the compounds that are discussed are being evaluated as part of a BL-BLI combination.

Expert opinion: The current trajectory in BLI development is promising; however, a significant challenge resides in the selection of an appropriate BL partner as well as the development of resistance linked to the BL partner. In addition, dosing regimens for these BL-BLI combinations need to be critically evaluated. A revolution in bacterial diagnostics is essential to aid clinicians in the appropriate selection of novel BL-BLI combinations for the treatment of serious infections.

Keywords

Antimicrobial resistance; β-lactam; β-lactamase; β-lactamase inhibitor; Gram-negative

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Declaration of interest

The author of this manuscript has or has had in the past two years research collaborations with Allecra, Entasis, Merck, VenatoRx, Wockhardt, Roche and Allergan. She has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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1. Introduction

β-Lactams (BLs) are the largest class of antibiotics. Their mechanism of action is to inhibit bacterial cell-wall synthesis by forming a stable adduct with the peptidase domain of penicillin-binding proteins (PBPs), thus stalling peptide crosslinking and resulting in cell death. There are four major classes of BLs: penicillins, cephalosporins, monobactams, and carbapenems. The most common BL resistance mechanism in Gram-negative bacteria is the production of β-lactamases or enzymes that hydrolyze the amide bond of β-lactams inactivating the antibiotic and its ability to inhibit PBPs. The most problematic and difficult-to-treat β-lactamase-producing Gram negatives include extended-spectrum β-lactamases (ESBL)-producing Enterobacteriaceae, carbapenem-resistant Enterobacteriaceae (CRE) that produce KPC- or OXA-48-like carbapenemases, *Pseudomonas aeruginosa*, and *Acinetobacter* spp. Based on their tertiary structures, four main groups of β-lactamases (classes A, B, C, and D) are circulating around the world [1–5]. Classes A, C, and D enzymes possess a nucleophilic serine residue that is required for BL hydrolysis; class B enzymes are metallo-β-lactamases that require Zn^{2+} for activity.

To evade the production of β-lactamases, β-lactamase inhibitors (BLIs) were discovered, and these molecules are given in combination with a partner BL, as most BLIs do not possess significant PBP inhibition on their own. BL-BLI combinations are referred to as β-lactam combination drugs by the Clinical Laboratory Standards Institute (CLSI). Clavulanic acid, sulbactam, and tazobactam were the first BLIs approved for use in the clinic; however, there BLI profiles are largely limited to class A serine penicillinases (e.g. TEM-1, SHV-1) and ESBLs (e.g. CTX-M-15) as well as some class C and D β-lactamases (e.g. AmpC and OXA-1) [6]. Correspondingly, due to the limited spectrum of these former BLIs as well as the spread of antimicrobial resistance in Gram-negatives due to the production of β-lactamases, novel BL-BLI combinations with expanded profiles were sought.

Three major chemical BLI scaffolds are represented in approved and developing BL-BLI combinations. β-Lactambased BLIs (sulfones and oxapenems) continue to have a presence. After decades of research, boronic acid BLIs (e.g., vaborbactam and taniborbactam) have reached the spotlight. New to the BLI space are diazabicyclooctane (DBO) BLIs (e.g., avibactam and relebactam), including DBOs with enhanced chemistries such that they can also target PBPs (e.g., durlobactam, zidebactam, and nacubactam). In addition, some older BL-BLI combinations were revamped as the partner BL was replaced (i.e., ceftolozane-tazobactam, cefepime-tazobactam, and ceftibuten-clavulanic acid). Another advancement in the BL-BLI field is a renewed focus on pharmacokinetics/pharmacodynamics – this topic will not be discussed in this review; thus, the reader is referred to two excellent contemporary reviews on this topic [7,8].

2. Recently-approved β-lactam-β-lactamase inhibitor combinations

Four BL-BLI combinations entered clinical use in the last 5 years. Ceftolozane-tazobactam, ceftazidime-avibactam, meropenem-vaborbactam, and imipenem-cilastatin-relebactam were approved by the Food and Drug Administration (FDA) to treat specific infections by certain Gram-negative pathogens (Table 1).

2.1. Ceftolozane-tazobactam

Ceftolozane-tazobactam was approved by the FDA for the treatment of complicated urinary tract infections (cUTI), including acute pyelonephritis and complicated intra-abdominal infections (cIAI) when combined with metronidazole in December 2014 in adults ≥ 18 years of age (Table 1) [9]. The combination was also approved for hospital-acquired bacterial pneumonia (HABP), and ventilator-associated bacterial pneumonia (VABP) for those ≥ 18 years of age and is being further evaluated for the treatment of infections in persons with cystic fibrosis and burns (clinicaltrials.gov identifiers: , and). Thus, the indications for treatment with ceftolozane-tazobactam may expand. Detailed reviews of ceftolozane-tazobactam are available [10,11].

Ceftolozane is a novel cephalosporin that was designed to be more stable to the class C *Pseudomonas*-derived cephalosporinase (PDC, also referred to as *Pseudomonas* AmpC) [12]. Ceftolozane possesses a reduced affinity for PDC and is not hydrolyzed (Figure 1 and Table 2). The BLI partner, tazobactam is a sulfonebased inhibitor with limited inhibitory activity against class A carbapenemases and class D oxacillinases [13,14]. Tazobactam was previously paired with the penicillin, piperacillin; ceftolozane-tazobactam appears to be more cost effective than piperacillin tazobactam due to improved quality-adjusted life years [15,16].

Ceftolozane-tazobactam's strength resides in its potent activity against *P. aeruginosa*, including multi-drug resistant (MDR) strains with 90–98% of contemporary isolates testing susceptible [17–26]. Moreover, ceftolozane-tazobactam is a potential carbapenem-sparing treatment regimen against ESBL-producing Enterobacteriaceae [18–22,24]. Ceftolozane-tazobactam's activity against ESBL-producing *K. pneumoniae* as revealed through susceptibility testing is largely lacking [20,21,24,27]; however, in spite of these *in vitro* results, clinical cure rates are high [28]. Conversely, the combination is mostly ineffective against Enterobacteriaceae with serine carbapenemases [29–31].

Since the introduction of the BL-BLI combination, resistance to the ceftolozane-tazobactam was reported during treatment and these resistance mechanisms were extensively explored [32–38]. The predominant resistance mechanism described is the acquisition of amino acid substitutions in PDC; these changes in select residues allow PDC to hydrolyze ceftolozane.

2.2. Ceftazidime-avibactam

In February 2015, ceftazidime-avibactam was approved by the FDA for the treatment of cUTIs, including acute pyelonephritis and cIAIs in combination with metronidazole for individuals ≥ 3 months of age (Table 1) [39]. Another clinical indication for the treatment of HABP/VABP was subsequently added for adults ≥ 18 years old [39]. The reader is directed to several recent ceftazidime-avibactam reviews with more in-depth information on the combination [40,41].

Ceftazidime is also a cephalosporin with an R1 side chain similar in structure to ceftolozane, but an aminothiazole replaces the aminothiadiazole (Figure 1 and Table 2). Avibactam is the first diazabicyclooctane (DBO) BLI to reach the clinic. Ceftazidime-avibactam is highly potent against Enterobacteriaceae carrying bla_{KPC} and bla_{OXA-48}; the MIC_{90} values reported

include 0.5 μg/mL for 24,750 isolates of Enterobacteriaceae and 2 μg/mL for a panel of >500 strains of CRE [42–45]. The activity of this combination extends to *P. aeruginosa* with a comparable percentage of isolates testing susceptible (96.8%) as with ceftolozane-tazobactam (99%) [46]. The spectrum of activity of ceftazidimeavibactam is attributable to avibactam's ability to inhibit class A, C, and some D β-lactamases, including KPC and OXA-48 carbapenemases [47,48].

Resistance to the ceftazidime-avibactam was observed during therapy as well as through *in vitro* screening, and the mechanisms leading to resistance were explored [49–53]. The principal mechanism described is the acquisition of amino acid substitutions (e.g. D179Y, V240G) in the KPC carbapenemase leading to enhanced catalytic efficiency (e.g. lower affinity or increased hydrolysis) toward ceftazidime. Other resistance mechanisms including membrane permeability and drug efflux were also found to influence ceftazidime-avibactam resistance.

2.3. Meropenem-vaborbactam

In August 2017, meropenem-vaborbactam was approved by the FDA for treatment of cUTI, including pyelonephritis in adults ≥ 18 years of age (Table 1) [54]. A Phase 1 clinical trial to test meropenem-vaborbactam in pediatric populations with bacterial infections is currently recruiting patients (clinicaltrials.gov identifier:). In addition, the combination completed Phase 3 clinical trials for the treatment of serious bacterial infections (e.g. HABP, VABP, and bacteremia) due to CRE in adults ≥ 18 years old (clinicaltrials.gov identifier:) [55]. Consequently, the indications for use of meropenem-vaborbactam may expand. Additional information on meropenem-vaborbactam is available in the following review articles [56–59].

Meropenem is a carbapenem and vaborbactam is a novel monocyclic boronic acid-based BLI and the first BL-boronate BLI combination to reach the market, which was highly anticipated due to the decades of research by many scientists on boronic acids and serine β-lactamases (Figure 1 and Table 2) [6]. Vaborbactam is an inhibitor of many class A and C β-lactamases, including KPC carbapenemases. Intriguingly, vaborbactam also demonstrates some inhibitory activity (IC_{50} values = 136–631 μM) against class B metallo-β-lactamases, including all three subclasses, B1, B2, and B3 [60]. The combination of meropenem-vaborbactam demonstrates potent antimicrobial activity against CRE with an MIC_{90} value of 1 μg/mL for meropenem when vaborbactam is maintained at 8 μg/mL [61,62]. Against a large panel (10,426 strains) of contemporary Enterobacteriaceae, meropenem and meropenem-vaborbactam possessed an MIC_{90} value of 0.06 μg/mL; however, against KPC producers the MIC_{90} values for meropenem and meropenem-vaborbactam differentiated to >32 μg/mL and 0.5 μg/mL, respectively [63]. Unlike ceftazidime-avibactam, vaborbactam does not potentiate the activity of meropenem against *P. aeruginosa in vitro* [64]. However, in a neutropenic murine thigh infection model with *P. aeruginosa*, meropenem-vaborbactam did reduce bacterial load; thus, the combination may have some utility against other Gram negatives [65].

In Enterobacteriaceae, resistance to meropenem-vaborbactam *in vitro* was attributed to the loss of expression of porins as well as increased expression of bla_{KPC} [66–68].

Ceftazidimeavibactam-resistant KPC-3 variants (e.g. V240G and D179Y) remained susceptible to meropenem-vaborbactam [68].

2.4. Imipenem-cilastatin-relebactam

In July 2019, imipenem-cilastatin-relebactam was approved by the FDA to treat of cUTIs and cIAIs caused by certain susceptible Gram-negative bacteria, in adults with limited or no alternative therapies available (Table 1) [69]. The combination is being further evaluated in Phase 3 trials for the treatment of infections in persons with HABP and VABP (clinicaltrials.gov identifier:). This carbapenem-DBO combination possesses antimicrobial activity against Enterobacteriaceae (MIC_{90} value = 0.5–1 μg/mL) and *P. aeruginosa* (MIC_{90} value = 2 μg/mL) producing class A and C β-lactamases (Figure 1 and Table 2) [70–72]. Relebactam is a potent inhibitor of KPC-2 and AmpC β-lactamases with $K_{i\ app}$ values of 2.3 μM and 3.4 μM respectively [73,74]. The imipenem-relebactam combination is effective at reducing bacterial load in neutropenic murine disseminated and pulmonary infection models caused by *P. aeruginosa* and Enterobacteriaceae [75]. Unlike ceftazidime-avibactam, the MICs of imipenem-relebactam are not effected by D179 variants of the KPC-2 carbapenemase [76]. However, loss of antimicrobial activity for imipenem-relebactam was reported in Enterobacteriaceae due to lack of expression of porins [77–80]. Interestingly, *oprD* mutants in *P. aeruginosa* are more susceptible to imipenem-relebactam; this result is likely due to the essentiality of bla_{ampC} expression in the *oprD* null background [80–82].

3. β-lactam-β-lactamase inhibitor combinations in development

Eight BL-BLI combinations are in various stages (Phase 1–3) of clinical development. These combinations include two BL-sulfone BLI combinations (i.e., cefepime-enmetazobactam and cefepime-tazobactam), several BL-DBO-BLI combinations (i.e., aztreonamavibactam, sulbactam-durlobactam, cefepime-zidebactam, meropenem-nacubactam, and cefpodoxime proxetil-ETX0282), and a BL-boronate-BLI combination (i.e., cefepime-taniborbactam). Multiple unique features exist for the BL-DBO-BLI combinations: dual-action, increased reactivity, and oral bioavailability.

Durlobactam, zidebactam, nacubactam, and ETX0282 possess dual PBP and β-lactamase inhibitor activity. These BLIs with β- lactam activity are occasionally referred to as β-lactam ‘enhancers’. When given in combination with a β-lactam partner, these DBOs not only inhibit the β-lactamases, but also target PBPs. These combinations work synergistically by targeting different PBPs at the same time, thus these DBOs ‘enhance’ the activity of the partner β-lactam. The chosen partner β-lactams for these DBOs, sulbactam, cefepime, meropenem, and cefpodoxime are potent PBP3 inhibitors resulting in the characteristic filamentation of the bacteria cell upon inhibition; conversely the DBOs inhibit PBP2 thus resulting in the formation of spheroplasts [83–85]. Together these BL-DBO combinations produced ‘spindle-shaped’ cells.

Using innovative chemistry, diazabicyclooctenone DBOs, durlobactam and ETX0282 were engineered with a double bond between C3 and C4 and methyl groups at the C3 position [85]. These modifications increased their reactivity as well as enhanced binding to β-lactamases. Predecessor DBOs, such as avibactam, lacked inhibitor activity against most

class D β-lactamases (e.g., OXA-23 and OXA-24/40). Consequently, this subclass of DBOs was rationally-designed using *in silico* approaches to expand the inhibition profile of DBOs to include class D β-lactamases.

Due to poor oral bioavailability, most BL-BLI combinations in development are only available in an intravenous formulation. IV drug administration is a critical route for drug delivery, but has associated risks and caveats (e.g., variable venous access, phlebitis, thrombophlebitis, infiltration, extravasation, infections, higher costs) [86,87]. Oral step-down therapy is beneficial as it eliminates the risks associated with IV administration and has the potential to decrease the length of hospital stay as well as improve quality of life for patients [88–94]. Cefopodoxime-ETX0282 is a novel oral BL-BLI combination in clinical trials.

3.1. Aztreonam-avibactam

Pfizer is developing the combination of aztreonam-avibactam, which will be entering Phase 3 clinical trials for the treatment of serious bacterial infections due to metallo-β-lactamase producing Gram negatives (clinicaltrials.gov identifier:) (Figure 2 and Table 2). Aztreonam, the only monobactam β-lactam approved for clinical use in the US, is stable to metallo-β-lactamases and by adding avibactam, the combination demonstrates antimicrobial activity against Enterobacteriaceae co-producing class B and A or C β-lactamases [95–97]. In neutropenic murine thigh infection models caused by metallo-β-lactamase producing Enterobacteriaceae and *P. aeruginosa*, aztreonam-avibactam lower the bacterial load [98]. Resistance to aztreonam-avibactam was reported in a panel of clinical isolates of *E. coli* producing $bla_{\text{NDM-1}}$ [99]. The mechanism of resistance was a four amino acid insertion into PBP3 abrogating the activity of aztreonam.

3.2. Cefepime-enmetazobactam

The cefepime-enmetazobactam combination is being developed by Allecra Therapeutics and is in Phase 3 clinical trials for cUTI (clinicaltrials.gov identifier:). The combination possesses potent activity against Enterobacteriaceae producing class A ESBLs and is a potential carbapenem-sparing treatment regimen [100,101]. Enmetazobactam is a penicillanic acid sulfone β-lactamase inhibitor, similar in structure to tazobactam (Figure 2 and Table 2). However, enmetazobactam possesses a methyl group on the triazole moiety that gives the molecule a neutral charge and is predicted to enhance entry into the bacterial cell as well as interactions with β-lactamases [102]. Enmetazobactam inhibits class A β-lactamases, including KPC carbapenemases with IC_{50} values ≤ 0.52 μM [102]. In murine neutropenic thigh infection and immunocompetent septicemia models using cefepime-resistant Enterobacteriaceae, the cefepime-enmetazobactam combination significantly reduced bacterial burdens [102,103]. According to the developer's website, enmetazobactam will likely be paired with piperacillin as well.

3.3. Sulbactam-durlobactam

Entasis Therapeutics is a pioneer in the development of antimicrobial niche therapy, their sulbactam-durlobactam (ETX2514) combination is slated to target MDR *Acinetobacter* spp. (Figure 2 and Table 2). This BL-BLI is in Phase 3 clinical trials for *A. baumannii*-

calcoaceticus complex HABP, VABP, and bacteremia (clinicaltrials.gov identifier:). This is the only BL-BLI combination in development that demonstrates potent antimicrobial activity against *Acinetobacter* spp., a formidable threat to public health [85,104]. Sulbactam is traditionally known as a BLI, however due to sulbactam's strong affinity for PBP3 in *Acinetobacter* spp., this BLI behaves as a BL [105]. Durlobactam inhibits class A, C, and D β-lactamases, thus is able to target the AmpC of *Acinetobacter* spp. (*Acinetobacter*-derived cephalosporinase, ADC) as well as the major groups of acquired oxacillinases (i.e., OXA-23-, OXA-24/40-, and OXA-58-families) in *Acinetobacter* spp [85,104]. Durlobactam also possesses β-lactam properties as it can inhibit PBP2 [85]. The sulbactam-durlobactam combination is effective in neutropenic murine thigh and lung infections models caused by MDR *Acinetobacter* spp. [85,104]. To identify potential resistance mechanisms to sulbactam-durlobactam, the combination and each drug alone were used to select for resistant mutants. With sulbactam, mutations in *pbp3* were identified that result in amino acid substitutions to the PBP3 active site and affect sulbactam binding [106]. Moreover, alterations in the bacterial stringent response occurred, which were correlated with durlobactam exposure [106].

3.4. Cefepime-tazobactam

The cefepime-tazobactam combination at a 1:1 ratio in development by Wockhardt Ltd will be entering Phase 3 clinical trials for cUTI and acute pyelonephritis (clinicaltrials.gov identifier:) (Figure 2 and Table 2). CLSI established susceptibility dose dependent (SDD) breakpoints (≤ 2 μg/mL up to ≤ 8 μg/mL) for cefepime that vary based on the chosen dose and infusion (0.5–2 grams every 8–12 hours) [107]. The addition of tazobactam will help cefepime cover isolates producing ESBLs that are resistant to piperacillin-tazobactam as well as strains with derepressed AmpCs [108,109]. Within the combination, cefepime is set at maximum dosage of 2 grams with 2 grams of tazobactam and is suggested to be administered every 8 hours as an extended infusion (90 min), thus allowing for broader coverage of isolates with higher cefepime-tazobactam MICs (8–16 μg/mL) [107,109]. Cefepimetazobactam demonstrates potent antimicrobial activity against Enterobacteriaceae, including those producing ESBLs with MIC_{90} values of 0.25 and 0.5 μg/mL when tazobactam if fixed at 4 and 8 μg/mL, respectively [107]. In addition, high-dose extended-infusion cefepime-tazobactam has potential applicability against KPC-producing Enterobacteriaceae [108,109].

3.5. Cefepime-taniborbactam

A novel cephem-bicyclic-boronate-BLI combination, cefepime-taniborbactam (VNRX-5133), which is entering in Phase 3 clinical trials for cUTI and acute pyelonephritis is in development by VenatoRx Pharmaceuticals (clinicaltrials.gov identifier:) (Figure 2 and Table 2). Taniborbactam potentiates the activity of cefepime against groups of Enterobacteriaceae producing KPC, VIM, NDM, ESBLs, and AmpCs, but not strains carrying IMP metallo-β-lactamases [110,111]. Moreover, cefepime-taniborbactam was found to be more potent than ceftolozane-tazobactam against a panel of *P. aeruginosa*, 70% vs. 56% of isolates tested susceptible, respectively [112]. Taniborbactam is a potent inhibitor of class A, B, C, and D β-lactamases with a K_i of 21.6 nM for the VIM-2 metallo-β-lactamase [113]. In addition, ceftazidime-avibactam-resistant KPC-3 variants and

ceftolozane-tazobactam-resistant PDC variants (except the E221K variant in the parent background) were susceptible to cefepime-taniborbactam [114]. The combination was found to be efficacious in a neutropenic murine lung infection model caused by cephalosporin-resistant *K. pneumoniae* and murine bacteremia and neutropenic-thigh infection models caused by carbapenem-resistant Enterobacteriaceae, including metallo-β-lactamase producers [115–117]. In Enterobacteriaceae, loss of porin production resulted in increased MICs to cefepime-taniborbactam; thus alterations in permeability impacts resistance to this combination [118].

3.6. Cefepime-zidebactam

Wockhardt, Ltd is developing the cephem-DBO combination of cefepime-zidebactam that has completed Phase 1 clinical trials (clinicaltrials.gov identifiers: , , ,) (Figure 2 and Table 2). Cefepime-zidebactam at a 1:1 ratio demonstrated antimicrobial activity against 5946 strains of Enterobacteriaceae and 1291 isolates of *P. aeruginosa* with reported MIC_{90} values of 0.12 and 4 μg/mL, respectively [119]. As zidebactam is a β-lactam 'enhancer', the cefepime-zidebactam combination also possesses activity against Enterobacteriaceae producing metallo-β-lactamases and class D oxacillinases and *P. aeruginosa* with metallo-β-lactamases [120–122]. Zidebactam, referred to as a bicyclo-acyl hydrazide on the basis of its chemical scaffold, inhibits class A and C β-lactamases and PBP-2 of *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* [84,123–125]. Despite poor *in vitro* activity (MICs 16–64 μg/mL) against *A. baumannii*, cefepime-zidebactam reduced bacterial burdens in neutropenic murine lung and thigh infection models with cefepime-resistant *A. baumannii* isolates [126,127]. The discrepancy in the *in vitro* and *in vivo* observations was likely due to the β-lactam 'enhancer' properties of zidebactam that alter the PK/PD properties of cefepime [128]. A similar observation was obtained with *P. aeruginosa* [129]. The eventual cefepime-zidebactam MIC susceptibility breakpoints will likely need to take these disagreements into account [130].

3.7. Meropenem-nacubactam

Meropenem-nacubactam is a carbapenem-DBO-BLI combination that completed Phase 1 clinical trials (clinicaltrials. gov identifier:) and is being developed by NacuGen Therapeutics, a joint venture between Fedora Pharmaceuticals and Meiji Seika Pharma (Figure 2 and Table 2). The addition of nacubactam restored meropenem susceptibility to Enterobacteriaceae including an isogenic panel of *E. coli* producing ceftazidime-avibactam-resistant KPC-3 variants [131–134]. As a result of meropenem-nacubactam's dual action, the combination also demonstrates reasonable activity (e.g. 71.2% of 309 isolates possessed an MIC of ≤1 μg/mL meropenem with 4 μg/mL of nacubactam) against metallo-β-lactamase producing Enterobacteriaceae [132,133,135]. Nacubactam inhibits PBP2 of Enterobacteriaceae and is a potent inhibitor of class A and C β-lactamases (IC_{50} < 1 μM) [83,136]. In neutropenic murine lung and cUTI infection models, meropenem-nacubactam was efficacious against Enterobacteriaceae-producing class A carbapenemases and Enterobacteriaceae-producing class A, B, C, or D β-lactamases, respectively [137,138]. Resistance to the nacubactam alone was attributable in most cases to global stringent response signal with induction of RpoS [139].

3.8. Cefpodoxime proxetil-etx0282

Cefpodoxime proxetil-ETX0282 is a novel oral cephem-diazabicyclooctenone combination in development by Entasis Therapeutics and is the first novel oral BL-BLI combination to reach Phase 1 clinical trials (clinicaltrials.gov identifier:) (Figure 2 and Table 2). The active components, cefpodoxime and ETX1317 demonstrate activity against Enterobacteriaceae and isogenic *E. coli* producing class A, C, and D β-lactamases [140–142]. In addition, ceftazidime-avibactam-resistant KPC-3 variants (V240G, D179Y, and D179Y/T243M) were susceptible to the combination [143]. ETX1317 inhibits class A, C and D β-lactamase with an IC_{50} values <0.54 μM and also binds to *E. coli* PBP2 [142]. The addition of ETX0282 to cefpodoxime reduced bacterial burdens in murine UTI and thigh infection models using ESBL-producing *E. coli* and KPC-2-producing *K. pneumoniae* [142,144].

4. Preclinical β-lactam-β-lactamase inhibitor combinations

Four novel BL-BLI combinations are in preclinical stages of development. These include a carbapenem-DBO combination, two oral-stepdown BL-BLIs, and a novel siderophore-cephem-DBO combination.

4.1. Meropenem-WCK 4234

WCK 4234 is a BLI with a DBO scaffold and a nitrile side chain and is in preclinical testing by Wockhardt Ltd in combination with meropenem under the name WCK 5999 (Figure 3 and Table 2). WCK 4234 is unique as it is the first molecule in the DBO class reported to meaningfully inhibit class D oxacillinases [123,145]. Correspondingly, at the time of its unveiling, WCK 4234 possessed superior inhibitory kinetic constants compared to avibactam and relebactam against class A and D β-lactamases [123]. Combined with meropenem, WCK 4234 lowered MICs against *A. baumannii* producing OXA-23, OXA-24/40 and OXA-51 carbapenemases [145]. Meropenem-WCK 4234 also demonstrated activity against Enterobacteriaceae producing KPCs and OXA-48-like β-lactamases [123,145]. The meropenem-WCK 4234 combination was not listed in the 2017–2018 Annual Report available on the developer's website; the status is uncertain.

4.2. Ceftibuten-clavulanate

A novel oral combination of ceftibuten-clavulanate, a cephem-oxapenem BLI was in preclinical development for ESBL-producing Enterobacteriaceae by Achaogen (Figure 3 and Table 2). Ceftibuten-clavulanate was the most active oral agent tested against a world-wide collection of 5,568 isolates of Enterobacteriaceae from 2017 [146,147]. Moreover, the pharmacodynamics of ceftibuten-clavulanate were evaluated in an *in vitro* chemostat model and a murine thigh infection model using ESBL-producing Enterobacteriaceae [148,149]. Unfortunately, in April 2019, Achaogen filed for bankruptcy, so the future of ceftibuten-clavulanate is unclear.

4.3. Ceftibuten-VNRX-7145

In preclinical testing by VenatoRx Pharmaceuticals, ceftibutenVNRX-7145 is an oral cephem-bicyclic boronate BLI combination that debuted at the American Chemical Society (ACS) National Meeting in 2019 (Figure 3 and Table 2) [150]. Ceftibuten combined with

VNRX-5236, the active molecule, demonstrates activity against Enterobacteriaceae producing class A, C, and D β-lactamases, including KPC and OXA-48 carbapenemases [151–154]. VNRX-5236 is a potent inhibitor of serine β-lactamases with an IC_{50} value of <0.5 μM for all tested enzymes [155]. The addition of VNRX-5236 to ceftibuten reduced bacterial burdens in neutropenic murine UTI and thigh infection models using ESBL- and KPC-2-producing *E. coli* and OXA-48 and KPC-producing Enterobacteriaceae, respectively [156,157].

4.4. GT-1-GT-055

The GT-1-GT-055 combination is a joint venture between Geom Therapeutics and LegoChem Biosciences and first debuted at American Society of Microbiology's Microbe meeting in 2018. GT-1 is a novel siderophore-based cephalosporin with an R1 side chain similar to ceftazidime, but with the addition of chloride to the aminothiazole (Figure 3 and Table 2). The introduction of the siderophore moiety enhances bacterial cell entry by allowing the drug to use the bacterial ferric iron transport system. GT-055 is a DBO-based BLI that also selectively binds to PBP2 in *E. coli* and *K. pneumoniae* (Figure 3) [158]. The GT-1-GT-055 combination possesses potent activity (MIC_{90} 2–4 mg/L) against a panel of Enterobacteriaceae producing class A, B, and C β-lactamases [159]. Against *P. aeruginosa* and *A. baumannii*, MIC_{90} values were 0.5–1 mg/L and 8 mg/L, respectively for GT1-GT-055 [160]. The combination also reduced bacterial loads ~1–2 logs below stasis in a murine thigh infection model with *K. pneumoniae* producing KPC-2 or GES-5 [161]. In Australia, Phase 1 clinical trials were discontinued in April 2019 which suggests that the development of this combination may have stalled (Australianclinicaltrials.gov identifier: ACTRN12618001980224).

5. Promising β-lactamase inhibitors

Based on their potent β-lactamase inhibitory activity, the MK-6183, QPX7728, ARX-1798, and BOS-572 BLIs are reviewed below despite lack of definitive or proposed β-lactam partners. Each of these BLIs has distinctive traits that fuel interest in further development.

5.1. MK-6183 (CB-238,618, CB-618)

MK-6183 is a DBO-based inhibitor that completed Phase 1 clinical trials in February 2019 (clinicaltrials.gov identifier:) (Figure 4). The clinical trial was initiated by the compound's former developer, Cubist; however, in 2015 Cubist was acquired by Merck and MK-6183 was transferred. To date, the pharmacokinetics-pharmacodynamics (PK/PD) relationship for MK-6183 efficacy in combination with various β-lactams against β-lactamase-producing Enterobacteriaceae was determined [162,163]. Based on their PK/PD observations, the authors suggest the MK-6183 may be useful as a 'standalone' BLI for the clinician to pair with the 'right' β-lactam [162]. The status of this compound is unknown as Merck's website does not list the drug in its pipeline.

5.2. QPX7728

In preclinical evaluation by Qpex Biopharma, QPX7728 is a novel bicyclic boronate-based BLI that was identified following *in silico* screening against serine and metallo-β-lactamases

(Figure 4) [164]. This BLI debuted at American Society of Microbiology's Microbe 2019 meeting in San Francisco, CA [165]. Combined with either aztreonam, ceftolozane, or meropenem, QPX7728 lowers MICs against Enterobacteriaceae, *A. baumannii* and *P. aeruginosa* producing class A, B, C, and D β-lactamases. QPX7728 also potentiated oral β-lactams, ceftibuten and tebipenem against CRE. QPX7728 possesses nM K_i values against purified class A, B, C, and D β-lactamases. In murine thigh and lung infection models, meropenem-QPX7728 lowered the bacterial load.

5.3. ARX-1796

ARX-1796 (AV-006) is an avibactam prodrug in preclinical testing by Arixa Pharmaceuticals (Figure 4). The charged sulfate moiety on avibactam limits its oral bioavailability; however, the addition of a neopentyl ester group to the sulfate enhances oral bioavailability in rats, dogs, and monkeys [166–168]. The oral β-lactams, ceftibuten, cefixime, amoxicillin, cefpodoxime, sulopenem, and tebipenem, were evaluated in combination with avibactam against a panel of Enterobacteriaceae producing ESBLs, KPCs, AmpCs, and OXA-48 and ceftibuten-avibactam demonstrated the lowest MICs overall [169,170]. Consequently, ARX-1796 may be partnered with ceftibuten.

5.4. BOS-572 (IID572)

Part of the DBO family, BOS-572 possesses a third ring that transforms this BLI into a dioxotriazatricyclohendecane (Figure 4). Originally discovered by Novartis, the compound is preclinical evaluation by Boston Pharmaceuticals. BOS-572 does not possess antibacterial activity on its own; thus, it must be combined with a β-lactam partner [171]. Combined with piperacillin, BOS-572 lowers MICs against an isogenic panel of *E. coli* carrying single class A, C, and D β-lactamases, including KPC-2 and OXA-48 carbapenemases [171]. The acylation rate (k_2/K_i) of BOS-572 is ~32x faster compared to avibactam against CTX-M-15 [171]. Relative to piperacillin-tazobactam with an MIC_{90} of >64 mg/L against 190 Enterobacteriaceae, piperacillin-BOS-572 possessed an MIC_{90} of 16 mg/L [171].

6. Conclusion

In this review, the latest advances in BL-BLI combinations were reviewed, including approved agents, ones in various stages of development (i.e., Phases 1–4), as well as BL-BLI combinations in preclinical testing. Significant progress was made in the past decade to develop BL-BLI combinations that target some of the most formidable Gram-negatives (ESBL-producing Enterobacteriaceae, CRE, *P. aeruginosa*, and *A. baumannii*). The spectrum of inhibition for these novel BLIs include class A, B, C, and D β-lactamases. Multiple BL-BLI combinations are carbapenem-sparing treatment regimens. Three oral stepdown BL-BLI combinations are in or close to the pipeline. Within the next decade, many of these new agents are anticipated to reach clinical use.

7. Expert opinion

A key finding in the field of BL-BLI development is the discovery of novel boronic acid- and DBO-based BLI scaffolds that inhibit the β-lactamases of today (e.g., KPC- and OXA-

carbapenemases). This advancement was critical in order to provide clinicians with alternative therapies to treat infections caused by CRE and MDR *P. aeruginosa*. Prior to this achievement, treatment options were limited to toxic agents, such as colistin. Another exciting development is the bicyclic boronates that inhibit metallo-β-lactamases. Between these BL-bicyclic boronate BLI combinations and aztreonam-avibactam, hopefully, the clinician will soon have an agent to use against Enterobacteriaceae-producing metallo-β-lactamases. A major advance in BL-BLI development is also oral step-down therapy, thus removing the risks associated with IV administration, reducing costs and improving the quality of life for patients.

Dosing regimens for these BL-BLI combinations need to be critically evaluated. For example, higher-dose extended infusion cefepime-tazobactam capitalizes on the safety of these two molecules. By increasing the dosage to the maximum, the spectrum of activity for this combination expands to potentially include CRE. However, caution is warranted, and the addition of therapeutic drug monitoring to clinical practice would likely enhance the utility of the antibiotic arsenal as well as reduce emergence of resistance.

A significant challenge exists in the selection of BL partners for these BLIs. Given what has been observed to date with ceftolozane-tazobactam and ceftazidime-avibactam, the BL partner is critical. Upon release of these two new BL-BLI combinations, resistance emerged during treatment. Single amino acid substitutions in the PDC and KPC β-lactamases were the main drivers behind resistance to these BL-BLI combinations. The PDC and KPC variants were more catalytically ‘competent’ against the partner BLs, diminishing their efficacy against PBPs when in combination. Are partner-less BLIs an option? MK-6183 was suggested to be useful as a ‘standalone’ BLI that could be then partnered pro re nata by an infectious disease clinician with a select BL to target different Gram-negatives. For example, MK-6183 paired with aztreonam may be effective against metallo-β-lactamaseproducing Enterobacteriaceae vs. MK-6183 partnered with imipenem may demonstrate activity against KPC-producing Enterobacteriaceae. However, such a practice would require intricate knowledge of the bacteria responsible for the infection as well as the resistance mechanisms present.

For the future, rapid methods to identify the bacterium and resistance mechanisms produced is critical toward choosing the correct antibiotic as well as preserving the antibiotics in our armamentarium. Molecular diagnostics in other fields has exponentially advanced; however, bacterial diagnostics remain trapped in the 1940s [172]. For true advancement, additional diagnostic tools are necessary. In fact, the challenge for the current Longitude Prize, which is a £10 million prize funded by several agencies in the United Kingdom, is to design an ‘accurate, rapid, affordable and easy to use’ point of care bacterial diagnostic test that will help combat antimicrobial resistance. Nevertheless, as we wait in anticipation, the latest advances in BL-BLI combinations are promising.

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Article highlights

- The development of novel BL-BLI combinations has escalated over the last decade.
- The spectrum of activity for 20 different novel BL-BLI combinations, including *in vitro* and *in vivo* studies, are presented; in addition, potential resistance mechanisms are described.
- The boronic acid- and diazabicyclooctane-based BLI scaffolds are likely the most outstanding advances in the field.
- The pursuit of novel ‘oral-stepdown’ BL-BLI combinations is fundamental necessity in the antibiotic arsenal.
- The greatest limitations in BL-BLI development are the selection of the BL partner, appropriate dosing strategies to obtain clinical cure, as well as rapid bacterial diagnostics to pinpoint the most suitable therapies.

This box summarizes key points contained in the article.

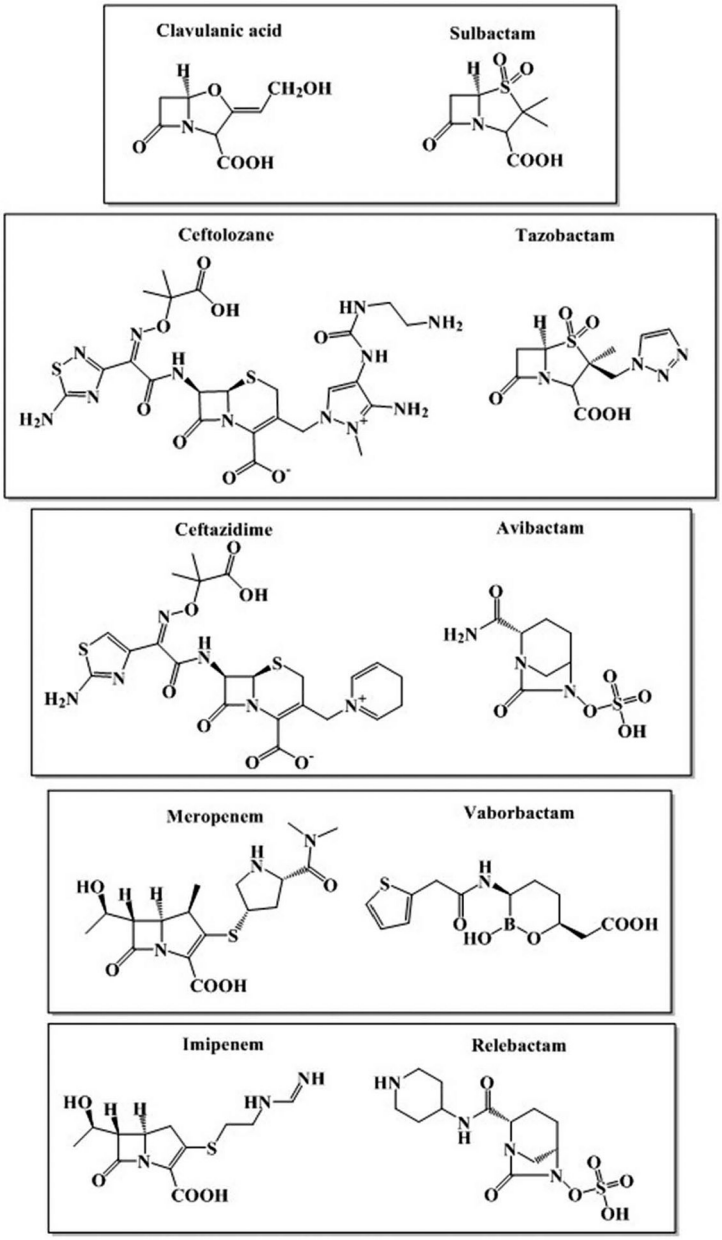

Figure 1.
FDA-approved BL-BLI combinations.

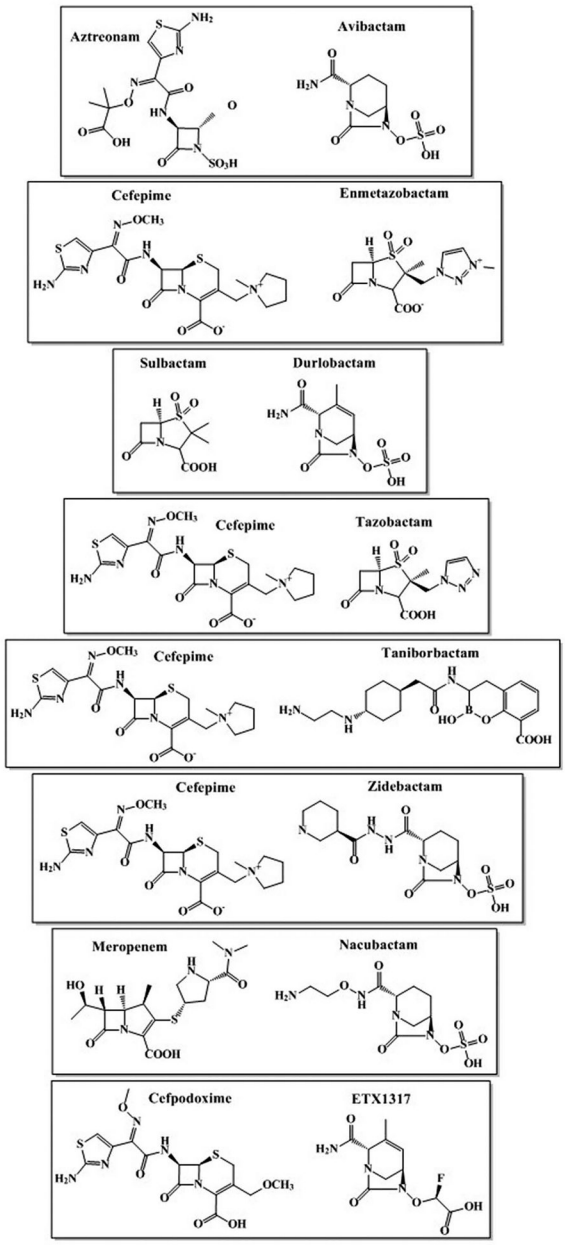

Figure 2.
BL-BLI combinations in clinical development.

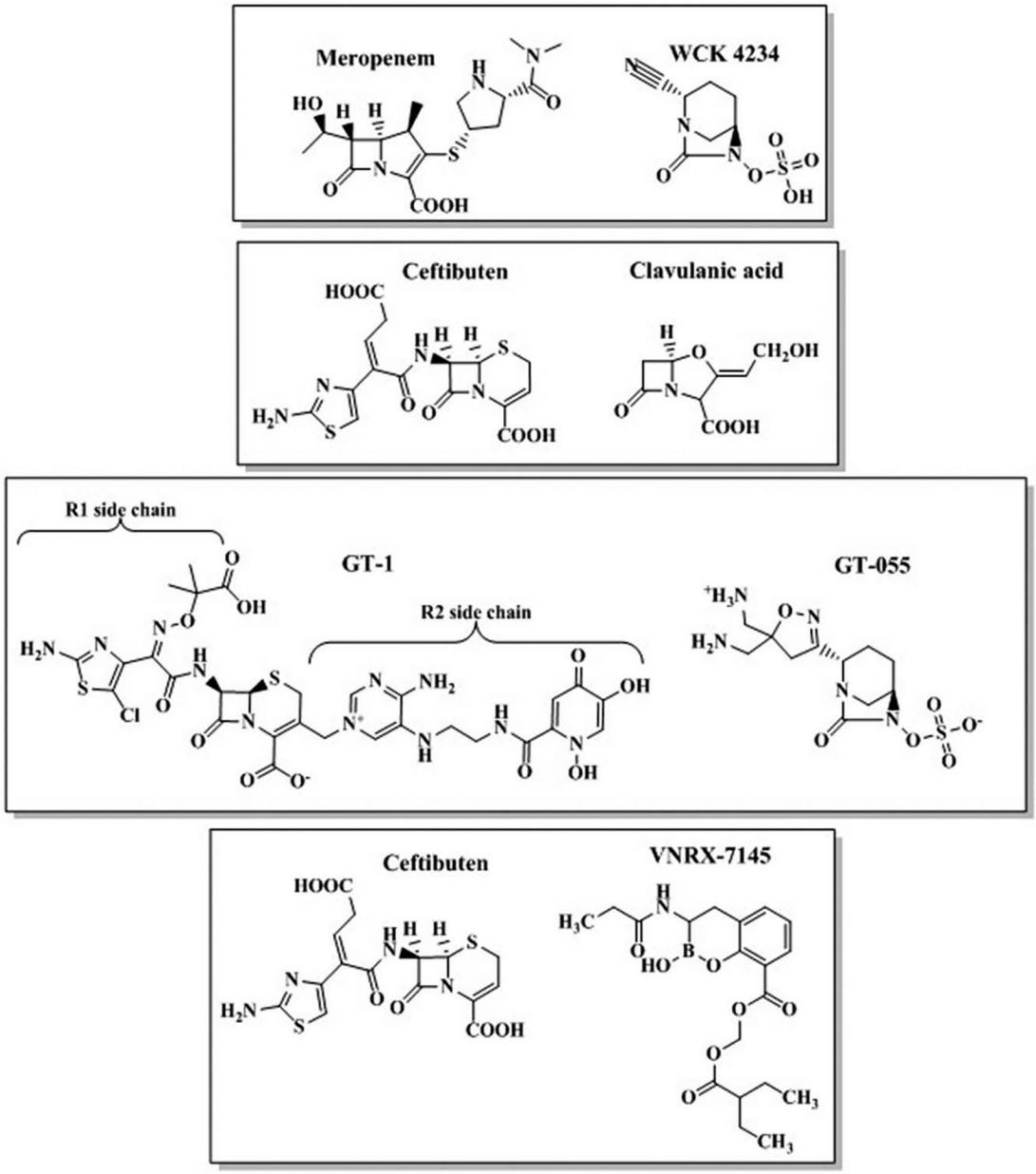

Figure 3.
BL-BLI combinations in preclinical testing.

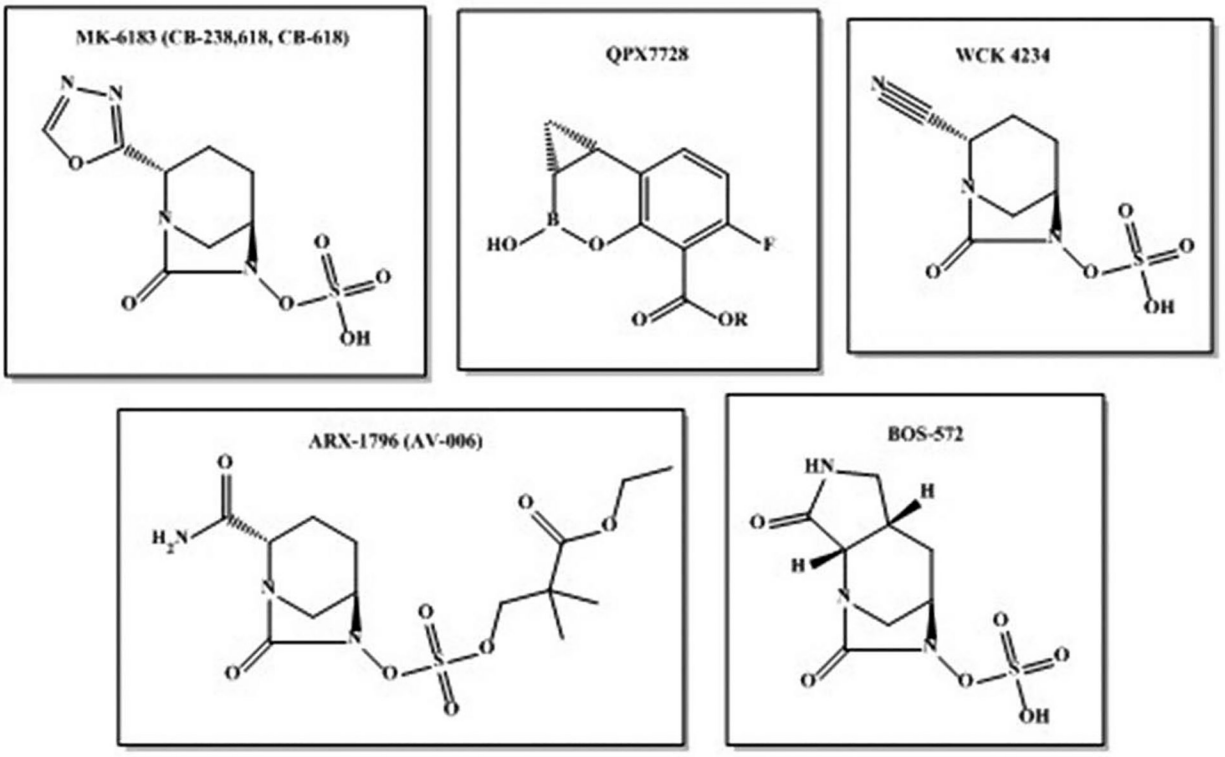

Figure 4.
BLIs in preclinical testing.

Table 1.

Indications and usage for FDA-approved BL-BLI combinations.

Drug	Approved Indication	Covered Gram-negatives	Population
Ceftolozane-tazobactam	cIAI in combination with metronidazole	*Enterobacter cloacae, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, P. aeruginosa, Bacteroides fragilis*	≥18 years
Ceftolozane-tazobactam	cUTI, pyelonephritis	*E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa*	≥18 years
Ceftolozane-tazobactam	HABP/VABP	*E. cloacae, E. coli, Haemophilus influenzae, K. oxytoca, K. pneumoniae, P. mirabilis, P. aeruginosa, Serratia marcescens*	≥18 years
Ceftazidime-avibactam	cIAI in combination with metronidazole	*E. coli, K. pneumoniae, P. mirabilis, E. cloacae, K. oxytoca, Citrobacter freundii* complex, *P. aeruginosa*	≥3 months
Ceftazidime-avibactam	cUTI, pyelonephritis	*E. coli, K. pneumoniae, E. cloacae, C. freundii* complex, *P. mirabilis, P. aeruginosa*	≥3 months
Ceftazidime-avibactam	HABP/VABP	*K. pneumoniae, E. cloacae, E. coli, S. marcescens, P. mirabilis, P. aeruginosa, H. influenzae*	≥18 years
Meropenem-vaborbactam	cUTI, pyelonephritis	*E.coli, Klebsiella. pneumoniae, E. cloacae species* complex	≥18 years
Imipenem-cilastatin-relebactam	cUTI, pyelonephritis	*E. cloacae, E. coli, Klebsiella aerogenes, K. pneumoniae, P. aeruginosa*	≥18 years
Imipenem-cilastatin-relebactam	cIAI	*Bacteroides caccae, B. fragilis, Bacteroides ovatus, Bacteroides stercoris, Bacteroides thetaiotaomicron, Bacteroides uniformis, Bacteroides vulgatus, C. freundii, E. cloacae, E. coli, Fusobacterium nucleatum, K. aerogenes, K. oxytoca, K. pneumoniae, Parabacteroides distasonis, P. aeruginosa*	≥18 years

Table 2.

Latest advances in β-lactam/β-lactam inhibitor combination for treatment of Gram-negative bacterial infections.

Combination	Type of BLI	Potential pathogens covered	Unique features	Development Phase
Ceftolozane-tazobactam	Sulfone	*P. aeruginosa*, Enterobacteriaceae-producing ESBLs	Targets *P. aeruginosa*, including MDR strains, carbapenem-sparing	FDA approved (2014)
Ceftazidime-avibactam	DBO	*P. aeruginosa*, Enterobacteriaceae-producing carbapenemases and ESBLs	First-in-class BLI, targets KPC and OXA-48-producing Enterobacteriaceae	FDA approved (2015)
Meropenem-vaborbactam	Boronate	Enterobacteriaceae-producing carbapenemases and ESBLs	First-in-class BLI, targets KPC-producing Enterobacteriaceae	FDA approved (2017)
Imipenem-cilastatin-relebactam	DBO	*P. aeruginosa*, Enterobacteriaceae-producing carbapenemases and ESBLs	Targets KPC-producing Enterobacteriaceae and *P. aeruginosa*	FDA approved (2019)
Aztreonam-avibactam	DBO	Enterobacteriaceae-producing carbapenemases, including metallo-β-lactamases	Activity against metallo-β-lactamase-producing Enterobacteriaceae	Phase 3
Cefepime-enmetazobactam	Sulfone	Enterobacteriaceae-producing ESBLs	Zwitterionic pair and carbapenem-sparing	Phase 3
Sulbactam-durlobactam	DBO	*A. baumannii-calcoaceticus* complex	Niche agent, targets *Acinetobacter* spp., and BLI possesses enhanced reactivity, is a β-lactam ‘enhancer’, and inhibits OXAs	Phase 3
Cefepime-tazobactam	Sulfone	Enterobacteriaceae-producing carbapenemases and ESBLs	High dose, extended infusion, carbapenem-sparing	Phase 3
Cefepime-taniborbactam	Boronate	Enterobacteriaceae-producing ESBLs, carbapenemases, including metallo-β-lactamases (except IMP)	Bicyclic boronate, activity against metallo-β-lactamases	Phase 3
Cefepime-zidebactam	DBO	*P. aeruginosa*, Enterobacteriaceae-producing carbapenemases, including metallo-β-lactamases and ESBLs. *Acinetobacter* spp.	Inhibits KPC- and metallo-β-lactamase- producing Enterobacteriaceae, BLI is a β-lactam ‘enhancer’ and bicyclo-acyl hydrazide	Phase 1
Meropenem-nacubactam	DBO	Enterobacteriaceae-producing carbapenemases, including metallo-β-lactamases and ESBLs	Inhibits KPC- and metallo-β-lactamase- producing Enterobacteriaceae, BLI is a β-lactam ‘enhancer’	Phase 1
Cefpodoxime-proxetil-ETX0282	DBO	Enterobacteriaceae-producing carbapenemases and ESBLs	Oral stepdown, and BLI possesses enhanced reactivity, is β-lactam ‘enhancer’, and inhibits OXAs	Phase 1
Meropenem-WCK 4234	DBO	Enterobacteriaceae-producing carbapenemases and ESBLs and *A. baumannii*	Inhibits KPC and OXA-48-producing Enterobacteriaceae and *Acinetobacter* spp., BLI inhibits OXAs	Preclinical
Ceftibuten-clavulanate	Oxapenem	Enterobacteriaceae-producing ESBLs	Oral stepdown, carbapenem-sparing	Preclinical
Ceftibuten-VNRX-7145	Boronate	Enterobacteriaceae-producing carbapenemases and ESBLs	Oral stepdown, inhibits KPC and OXA-48-producing Enterobacteriaceae	Preclinical
GT-1-GT-055	DBO	*P. aeruginosa*, Enterobacteriaceae-producing carbapenemases, metallo-β-lactamases, and ESBLs and *A. baumannii*	Siderophore-cephem and broad spectrum activity; inhibits KPC- and metallo-β-lactamase- producing Enterobacteriaceae,	Preclinical