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Recently developed synthetic compounds with anti-infective activity

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The ability of antibiotics to cure bacterial infections is at a serious risk due to the emergence and worldwide spread of superbugs. A lack of innovation and investment for almost 50 years has led to significant efforts currently being devoted to find alternative and innovative therapies to face this challenge. This short review highlights some of the recent efforts to develop synthetic small molecules with anti-infective activity. This article is focused on those compounds that, when co-administered with an antibiotic, enhance the antimicrobial action of the drug, as well as compounds that target unexplored objectives for bacterial survival. Selected examples are provided.

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Introduction

Antibiotics are probably the drugs that have transformed modern medicine the most. These drugs have managed to: (i) cure diseases that were fatal in the past, (ii) contribute greatly to increased life expectancy, and (iii) manage common infectious complications in vulnerable patients undergoing treatment. In all of these cases, the ability to treat secondary infections is crucial for patient recovery. Unfortunately, the ability of these drugs to cure infections caused by bacteria is now at serious risk due to the emergence and worldwide spread of superbugs (multi-resistant) [1]. Of particular concern is the increasing incidence in healthcare-associated systems, since in these cases the weak immune systems of patients facilitate the pathogenicity of bacteria. Resistance to antibiotics is reaching such dangerous levels that the World Health Organization (WHO) estimates that by 2050 around 10 million people could die every year as a result of this problem, and deaths from antibiotic resistance will exceed those caused by cancer.

Although bacteria will always be resistant due to their adaptability and intrinsic evolutionary character to develop highly efficient resistance mechanisms to escape the action of antibiotics, we must have solutions to keep them under control [2]. To this end, given the gap in investment in R&D by the big pharmaceutical companies since the 1960s and the small number of innovative approaches employed, which were mainly focused on improving existing drugs, anti-infective discovery strategies are currently focused on two approaches: (i) the development of antibiotic adjuvants for combined therapy with the existing antibiotics in clinical use; and (ii) the discovery of small molecules with new mechanisms of action that can disable unexplored objectives for bacterial survival [3••]. This short review highlights some recently described synthetic small molecules with anti-bacterial activity in the context of the two strategies outlined above.

Antibiotic adjuvants – β-lactamase inhibitors

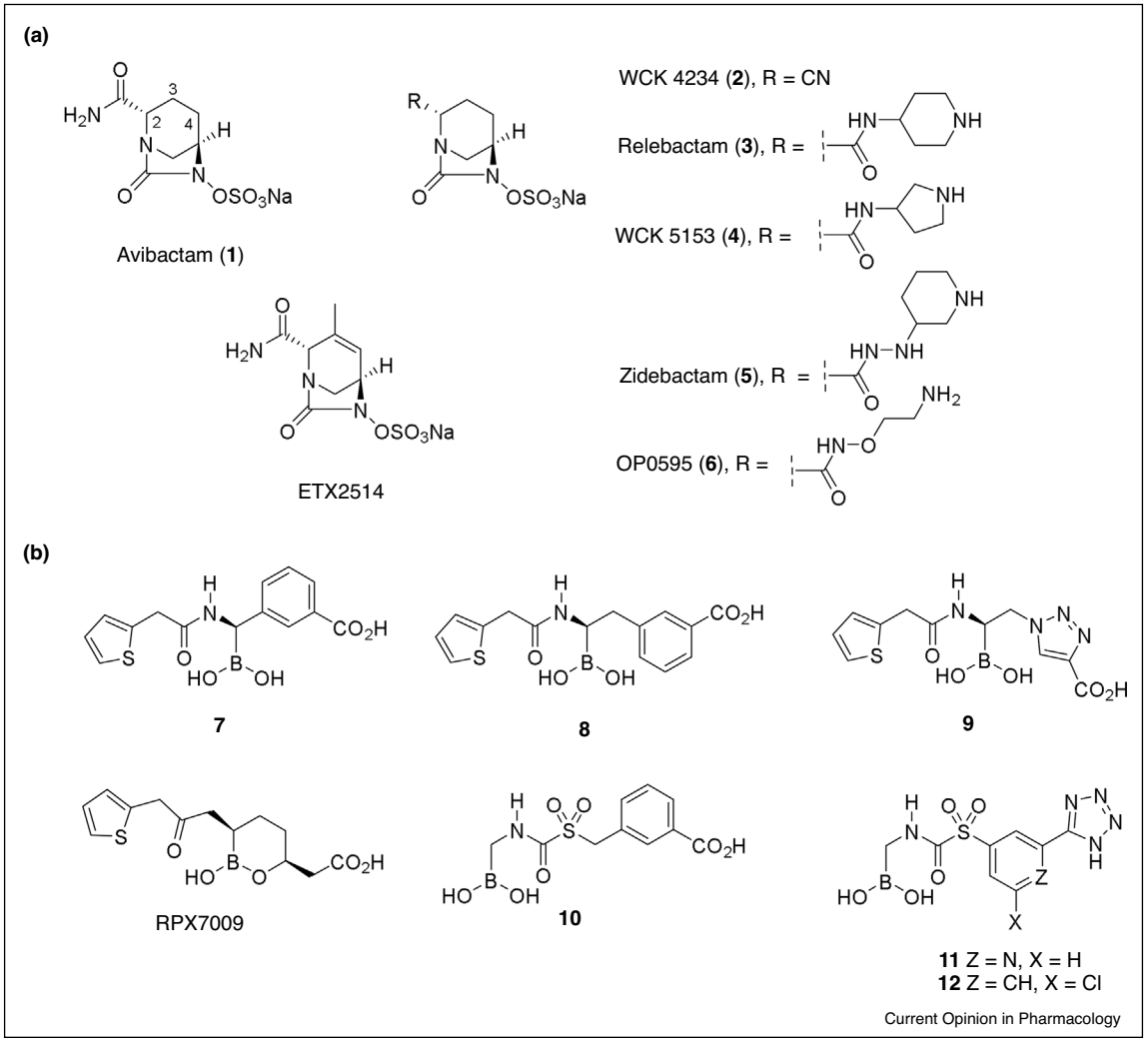
Antibiotic adjuvants, also named resistance breakers or antibiotic potentiators, are compounds that do not inhibit bacterial growth in their own right but when co-administered with the antibiotic they enhance the antimicrobial action of the latter [4–6,7••,8–10,11•]. Adjuvants breathe new life into antibiotics that have saved millions of lives for years but are now inefficient against superbugs. The most remarkable antibiotic adjuvants are those that block the main bacterial resistance mechanism to β-lactam antibiotics, that is, enzymatic inactivation of the drug by hydrolysis of the β-lactam core in an acylation–deacylation-based process catalyzed by β-lactamases enzymes. Among the four known types of β-lactamases (A–D), the most worrisome ones are the class D β-lactamases (oxicillinases, OXA) because they can inactivate the entire spectrum of β-lactam antibiotics, penicillins, cephalosporins, and even carbapenems, which are the antibiotics of last resort [12••]. These β-lactamases are widespread among the multi-resistant healthcare-associated infections caused by the Gram-negative ESKAPE pathogens, such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Enterobacteriaceae*, which were designated in 2017 by the WHO as the top priority pathogens for the development of novel anti-infective therapies [13]. The β-lactamase inhibitors in clinical use, namely clavulanic acid, sulbactam, and tazobactam, are ineffective against

class D β-lactamases and much effort has, therefore, been devoted to the development of more effective chemical entities, with some examples already in clinical studies. These compounds fundamentally fall into two categories: (1) diazabicyclooctanes and (2) boronic acids (Figure 1).

Diazabicyclooctanes (DBOs)

These are bicyclic compounds that undergo ring opening of their urea core by the catalytic serine to afford a stable carbamoyl adduct. The most representative example is avibactam, which was approved in 2014 by the FDA in combination with ceftazidime and is actually in clinical studies in combination with other antibiotics (Figure 1a) [14,15]. Avibactam has a unique mechanism of inhibition among the β-lactamase enzymes since it proved to be a covalent and slowly reversible inhibitor [16–18]. It has also been shown that avibactam targets penicillin-binding protein 2 in *Escherichia coli*. The main limitation of this compound is its variable inhibitory capacity against carbapenem-hydrolyzing class D β-lactamases, in particular OXA-24/40 and OXA-23, which represent the most prevalent and dangerous examples in the WHO top priority pathogens. The latter effect is due to the uncommon geometry of the active site, which has a tunnel-like entrance formed by Tyr/Phe and Met residues that act as a hydrophobic filter to allow the entrance of only certain substrates. In an effort to extend the avibactam spectrum activity, the DBO scaffold has been modified either by introducing other functional groups in position C2 or by functionalizing the cyclohexane core in positions C3 and C4. For example, Durand-Réville *et al.* [19] reported that the introduction of a double bond between positions C3 and C4 of avibactam and the inclusion of a methyl group in C3, that is, compound ETX2514, enables effective inhibition of the most dangerous OXA enzymes in *A. baumannii*, OXA-24/40 and OXA-23. In combination

Figure 1



Most relevant β-lactamase inhibitors. **(a)** Diazabicyclooctanes. **(b)** Boronic acids.

with piperacillin, ETX2514 (4 μg/mL) shows MIC values of 4 and 2 μg/mL against OXA-24/40 and OXA-23 from *A. baumannii*, respectively, while avibactam has poor *in vitro* activity for both enzymes (MIC > 64 μg/mL). More importantly, the combination of sulbactam/ETX2514 proved to have excellent *in vitro* activities of 0.5 μg/mL for both enzymes. The resolution of the crystal structure of OXA-24/40 from *A. baumannii* in complex with ETX2514 (PDB entry 5VFD) revealed that the aforementioned modifications in the avibactam scaffold enhance apolar interactions with the tunnel-like entrance and this explains the increase in activity. ETX2514 is now in phase I clinical studies. Moreover, Papp-Wallace *et al.* [20] showed that the replacement of the primary amide group in C2 by other more complex amide groups, specifically compounds WCK 5153, relebactam, zidebactam (WCK 5107), or by a nitrile group (WCK 4234), enhances the *in vitro* activity against OXA-24/40 and OXA-23 from *A. baumannii* by up to 64-fold. In addition, this enhancement is more pronounced in other OXA enzymes such as KPC-2 or OXA-48, both from *Klebsiella pneumoniae*.

Boronic acids

These compounds are mimics of the tetrahedral intermediate obtained after nucleophilic attack of the catalytic serine of the β-lactamase enzyme to the β-lactam core of the antibiotic (Figure 1b). Relevant examples are compounds **7**–**9**, which contain the thiophen-2-yl group of the natural penicillins and a carboxylate moiety to interact with the carboxylate binding pocket [21–26]. The resolution of diverse crystal structures of the corresponding enzyme adducts provides a good understanding of the potency of these ligands. The use of fragment-based design subnanomolar inhibitors led to the identification of compounds **10**–**12**, which have good *in vivo* antibacterial activity [27]. Acyclic boronic acids also proved to be good inhibitors for both metallo-β-lactamases and serine-β-lactamases [28,29]. The best example is RPX7009, which is in phase 3 clinical trials [30].

Small molecules that target unexploited objectives for bacterial survival

In general, the mode of action of antibiotics in clinical use is based on the prevention of the synthesis and assembly of key components for bacterial survival (bacterial viability), the inhibition of cell wall biosynthesis, DNA replication, RNA transcription, the biosynthesis of folates or the biosynthesis of proteins. Although this strategy is very effective and has given rise to a good arsenal of life-saving compounds, all of them inhibit a reduced number of biological targets and resistance to them is well known and widespread. It is not surprising, therefore, that there is great interest in exploring other bacterial functions and developing compounds with new mechanisms of action. Two examples of pathways that have attracted significant attention are highlighted below.

Inhibitors of the lipid A biosynthesis

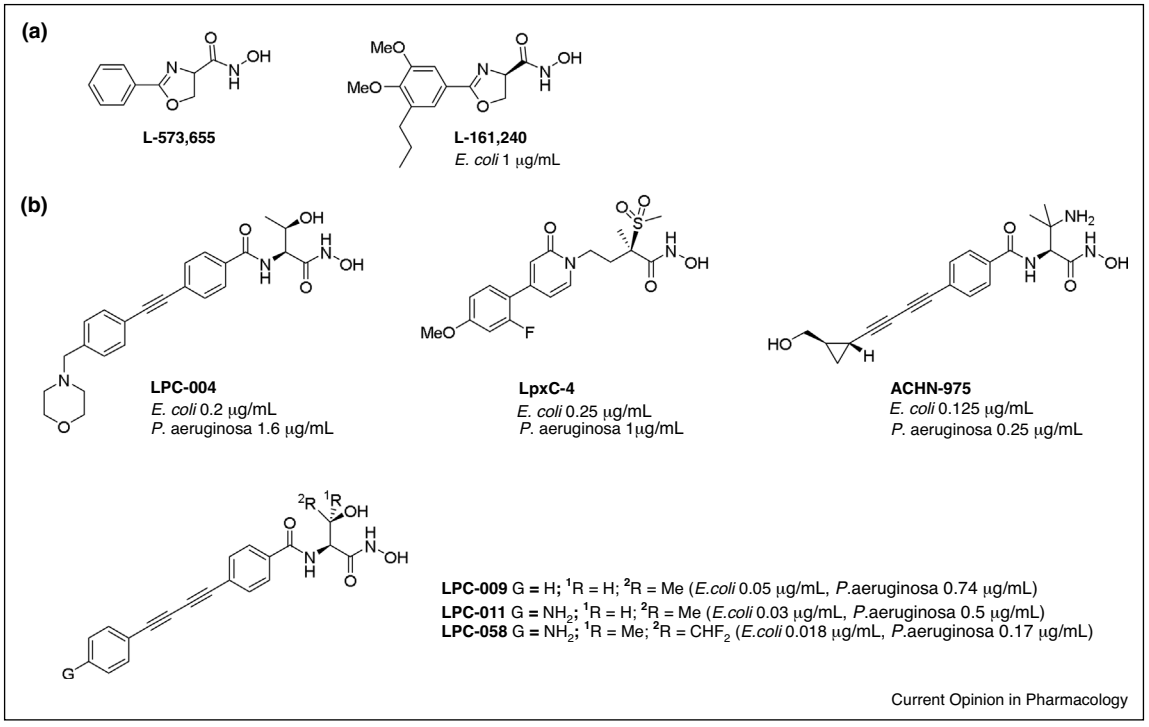
The enzymes of the lipid A pathway are attractive targets for Gram-negative anti-infective drug discovery because lipid A is: (i) the main component of the outer membrane of the Gram-negative bacteria, which differentiate themselves from the Gram-positive ones; (ii) essential for bacterial survival in relevant pathogens such as *P. aeruginosa* or *E. coli*; and (iii) involved in the capacity of the Gram-negative bacteria to cause infection. Among the enzymes involved in the pathway, only the LpxC enzyme, which catalyzes the second step of the route, has been studied and several inhibitors are already in clinical trials. The identification of the oxazoline hydroxamic acid L-573,655, followed by its improved version L-161,240, a hydroxamic acid with the *R* configuration, triggered all of the subsequent studies in this area [31•]. L-161,240 is a reversible competitive inhibitor of the *E. coli* enzyme with a K_i value of 24 μM and MIC values against *E. coli* up to 1 μg/mL. As both L-573,655 and L-161,240 are ineffective for *P. aeruginosa* treatments, efforts were devoted to the development of novel chemical entities suitable for this pathogen. The most relevant inhibitors reported are summarized in Figure 2 and they all have a hydroxamic acid with the *R* configuration linked to a long aliphatic tail that mimics the (*R*)-3-hydroxymyristoate moiety of the natural substrate [32–41]. These LpxC inhibitors have excellent *in vitro* activities against both *E. coli* and *P. aeruginosa*. Among them, ACHN-475 is already in clinical trials. The binding mode of these inhibitors has been well established with the resolution of a wide range of LpxC crystal structures from *P. aeruginosa*, *E. coli*, *Aquifex aeolicus* and *Yersinia enterocolitica* in complex with these compounds [42]. It is important to highlight that the aforementioned inhibitors, and in general the LpxC inhibitors, do not inhibit the growth of *A. baumannii* (MIC > 512 μg/mL), another critical Gram-negative pathogen reported by the WHO, since lipid A is not essential for this bacterium [43].

Inhibitors of the shikimic acid pathway

The enzymes involved in the shikimic acid pathway have attracted a great deal of attention for the development of new anti-tubercular therapies since six of the seven enzymes in the route are essential for *Mycobacterium tuberculosis* – the causative agent of tuberculosis – and they do not have any counterpart in human cells [44]. Four of the enzymes in the pathway are also essential for *Helicobacter pylori*, the causative agent of gastric and duodenal ulcers and also classified as a type I carcinogen, and, therefore, inhibitors that disable these targets have also been reported.

In accordance with the mechanism of action of the type II dehydroquinase, which catalyzes the third step, a large number of competitive reversible inhibitors have been reported that mimic the enolate intermediate involved [44]. As a carboxylic group in the inhibitor is required in

Figure 2



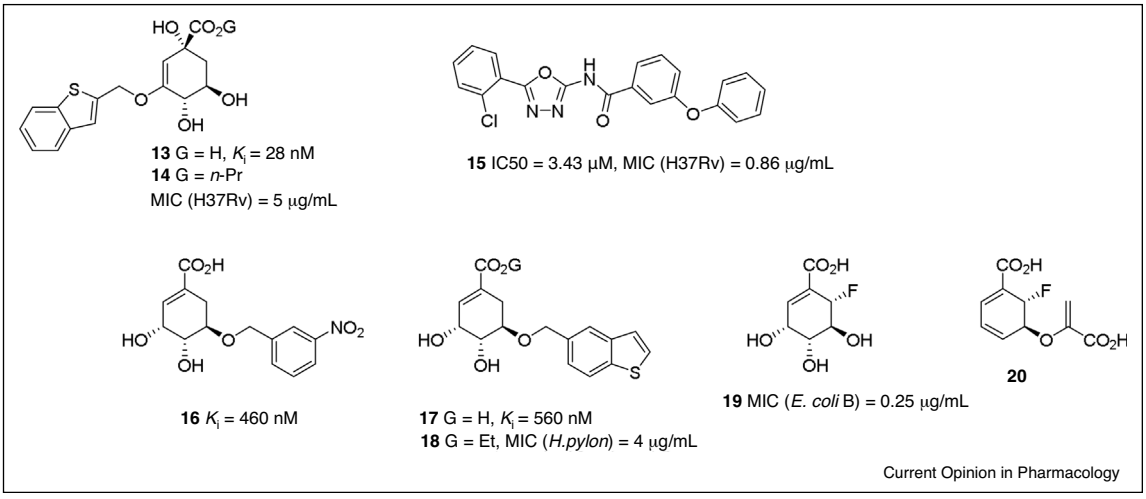
Most relevant LpxC inhibitors. **(a)** First examples. **(b)** Most potent inhibitors reported. MIC values against *E. coli* and *P. aeruginosa* are also included.

order to achieve good affinity for this enzyme, since it is a key point for recognition for all enzymes in the pathway, obtaining good *in vitro* activities has been the bottleneck for many of the developed inhibitors. Considering that the low *in vitro* activity obtained could be due to the high hydrophilicity of the compounds, lipophilic prodrugs (ester prodrug form) were designed. In principle, these ester derivatives would be slowly hydrolyzed to the carboxylate active form after absorption by the bacterium (cytosol). Fortunately, the *in vitro* activity dramatically increases with the stability of the ester against hydrolysis, proving to be the propyl ester derivatives the most efficient ones. Compound **14** was the most active example, with an MIC value of 5 μg/mL, and its active form compound **13** gave a K_i of 28 nM (Figure 3) [45]. The resolution of the crystal structures of the DHQ2 enzyme from *H. pylori* and *M. tuberculosis* in complex with these types of compounds revealed that the aromatic moiety freezes the substrate-covering loop, which contains two essential residues, in an inactive conformation for catalysis. Thus, this moiety interacts with the catalytic tyrosine of the loop by π-stacking and expels the catalytic arginine from the active site [45,46].

From a library of about 400 anti-mycobacterial compounds previously identified by the NIH Tuberculosis Antimicrobial Acquisition and Coordination Facility (TAACF), Simithy *et al.* [47] identified an inhibitor of shikimate kinase from *M. tuberculosis*, the fifth enzyme of the pathway, namely the oxadiazole-amide **15**, which had an MIC value of 0.86 μg/mL and an IC_{50} value of 3.43 μM with the isolated enzyme. Moreover, considering the large conformational changes required for the shikimate kinase enzyme in the LID and shikimic acid binding domains for product release, diverse C5-substituted shikimic acid analogs were developed to stabilize an inactive open conformation of the enzyme [48]. The 3-nitrobenzyl (**16**) and 5-benzothiophenyl (**17**) derivatives proved to be the most potent inhibitors, with K_i values of 460 nM and 560 nM, respectively. Ethyl ester **18** (a proform of **17**) was the most efficient derivative in achieving good *in vitro* activity against *H. pylori* and this had an MIC value of 4 μg/mL.

Zeneca Pharmaceuticals discovered that (6*S*)-6-fluoroshikimic acid (**19**), a fluorinated analog of the natural substrate of shikimate kinase, inhibited the growth of *E. coli* B with an MIC value of 0.25 μg/mL [49]. The antibacterial activity of **19** is due to the irreversible inhibition of 4-amino-4-deoxychorismate synthase by 2-fluorochorismic acid (**20**) [50]. The latter compound is generated *in vivo* from **19** by the last three enzymes of the pathway, specifically shikimate kinase, EPSP synthase and chorismate synthase.

Figure 3



Most relevant inhibitors of the shikimic acid pathway with antibacterial activity.

Conclusions and outlook

After a prolonged and incomprehensible lethargy, the future of the discovery of new anti-infective agents is compelling. In the foreseeable future, combination therapy strategies will probably be the most successful since (i) they do not require the identification and validation of new therapeutic targets; and (ii) they also allow us to preserve and/or rescue drugs that have been in use for years but are now less effective. This is perhaps why such compounds are the most common in the still limited new treatments in clinical studies. However, the development of compounds with new mechanisms of action, despite the challenges and the cost, can dramatically expand our ability to control bacteria. This approach will provide new weapons to deal with this significant problem. The recent progress is already very significant, as shown by the examples discussed here.

Conflict of interest statement

Nothing declared.

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