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Inhibitors of antibiotic resistance mechanisms: clinical applications and future perspectives

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“Antivirulence strategies can be considered a promising approach to overcome the global threat of antibiotic resistance as this allows you to deprive the bacterium of its pathogenicity without interfering with its life cycle.”

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Bacterial strains responsible for antibiotic resistant infections are increasing in an alarming way and the evolution of resistance mechanisms seems to be unstoppable. In the past decade, many efforts have been made in order to counteract this phenomenon but very few compounds have reached clinical trials. The development of new classes of antibiotics able to overcome the main bacterial drug resistance mechanisms is urgently required to counter the imminent danger of a postantibiotic era [1].

Scientific research should be oriented toward the discovery of new antimicrobial agents with specific features, including the capability to kill pathogens selectively without affecting the microbiota and to interfere with important virulence factors such as the biofilms [2]. Therapeutic strategies targeting virulence factors without interfering with microbial growth were recognized as a valuable alternative to conventional antibiotics as they should impose lower selectivity pressure on the rise of antibiotic-resistance strains [3]. Despite the many advances that have been made in this field, the lack of *in vivo* experiments to assess the clinical potential of the new antivirulence agents still remains one of the main drawbacks.

Serious chronic infections, especially in hospital settings, are frequently caused by six bacterial pathogens known with the acronym of their names, ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.). Understanding the mechanisms of antimicrobial resistance in these pathogens is crucial for identifying new strategies to treat drug-resistant infections.

The biochemical mechanisms of antibiotic resistance such as enzymatic resistance, which causes the inactivation of the antibiotics and efflux pumps responsible for the extrusion of the drug from the bacterial cell, were considered valuable drug targets for add-on treatment of bacterial infections.

The expression of the efflux pumps could be considered one of the main mechanisms of resistance against antibiotics in both Gram-positive and Gram-negative pathogens.

In the past decade, many efforts have been made to develop new potent efflux pump inhibitors (EPIs). A wide number of chemical compounds belonging to various chemical families, including quinolines, quinolones, naphthene compounds, boronic acid derivatives, chalcones and indole derivatives, have been described for their significant efflux pump inhibition activity. Representative examples of synthetic small molecules with EPI activity are the naphthene derivatives, described by Thota *et al.* as 3-(3,4-dihydronaphthalen-2-yl)-propenoic acid isobutyl and N,N-diisopropylamide amides, which act as NorA inhibitors reducing the minimum inhibitory concentration (MIC) value of ciprofloxacin (CPX) from 8 to 0.5 µg/ml against NorA overexpressing *S. aureus* 1199B [4]. Among boronic acids, Fontaine *et al.* identified 6-benzyloxyipyridine-3-boronic acid and 4-benzyloxyphenyl boronic acid as

new promising NorA inhibitors, which showed a significant synergistic activity with CPX against *S. aureus* 1199B with MMC₄ (minimum modulatory concentration of inhibitor to achieve a fourfold CPX MIC reduction) values of 1 and 0.5 µg/ml, respectively [5].

Even though the combination of EPIs and antibiotics was associated with an improvement of the antibacterial activity and the antimicrobial spectrum of antibiotics, no EPIs reached the clinic, despite a promising topical use of such inhibitors. This is due to their high toxicity and low *in vivo* efficacy [6].

Moreover, an ideal candidate as a new antimicrobial agent should be active against the planktonic form of life, as well as active against the biofilm phenotype. Biofilms are currently considered one of the most important bacterial virulence factors, which are responsible for serious nosocomial infections resistant to conventional antibiotics [7]. Structurally, biofilms are microbial communities in which the bacterial cells are enclosed into a matrix constituted of extracellular polymeric substance such as polysaccharides, proteins, lipids and extracellular DNA (e-DNA) [8].

Despite the growing knowledge of the biofilm organization and the important results obtained in the past decade in this research field, the treatment of biofilm-associated infections remains an important challenge. Recently, many compounds endowed with potent *in vitro* antibiofilm activity were described [9–11], but, unfortunately, no derivative is close to clinical trials. This is generally due to the lack of *in vivo* experiments aimed to confirm their antivirulence properties and their protective effects against infections caused by bacterial-resistant strains.

Three main mechanisms of action are identified as responsible for antibiofilm activity: the inhibition of the bacterial adhesion to surfaces, which is the first step of bacterial pathogenesis, the disruption of the biofilm architecture during its maturation process and the interference with the quorum sensing system [12,13].

The most relevant targets involved in the antibiofilm activity are: quorum sensing signalling molecules such as *N*-acylhomoserine lactone in Gram-negative pathogens, the autoinducing peptides described in Gram-positive bacteria and autoinducer-2 which was found in both Gram-negative and Gram-positive bacteria; nucleotide second messenger signaling systems including cyclic dimeric guanosine monophosphate and the cyclic dimeric adenosine monophosphate [14]; a modified nucleotide, tetra and pentaphosphate guanosine derivative of GTP and GDP known as (p)ppGpp and finally, targets concerning the microbial attachment to the surfaces such as the transpeptidase Sortase A for Gram-positive pathogens and type 1 fimbriae for Gram-negative strains [2].

Many classes of compounds were described in the past decade for their interesting antibiofilm properties, including imidazole compounds, indoles, carbazoles, phenazine and quinoline derivatives. Of these, the imidazole scaffold was the most investigated. The most significant contribution was given by Melander and coworkers who synthesized several libraries of 2-aminoimidazole derivatives that are able to prevent biofilm formation or disperse mature biofilms at low micromolar concentrations through mechanisms which do not affect microbial growth, therefore showing a typical antivirulence profile [15].

Another relevant class of heterocyclic compounds was represented by indole derivatives, which are widely described for their numerous biological activities, in particular, for the anticancer [16,17] and antibacterial [11] properties. Indole 5-haloaldonitrone were described as NorA inhibitors with synergistic effect with CPX against SA-1199B showing MIC values in the range 0.5–2 µg/ml in combination with subinhibitory (MIC/4) concentrations of the antibiotic [18].

Despite important progress that has been made to counteract the antibiotic resistance, including the understanding of the main molecular mechanisms at the base of the onset of resistant bacterial strains, we are still very far from identifying an effective and safe strategy that can reach the clinical phase. Different aspects must be taken into consideration to identify reliable candidates to fight multi-drug-resistant (MDR) strains. In the case of new compounds interfering with the microbial viability, the studies on the spontaneous frequencies of resistance were particularly relevant as they allow for the establishment of the capability of the compounds to promote antibiotic resistance and their suitability to be developed into new antibacterial drug.

Antivirulence strategies can be considered a promising approach to overcome the global threat of antibiotic resistance as this allows you to deprive the bacterium of its pathogenicity without interfering with its life cycle. Among the virulence factors, bacterial biofilms certainly represent one of the most valuable targets. *In vivo* studies aiming to validate new antibiofilm drugs are urgently needed for counteracting the emergence of chronic biofilm-associated infections.

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