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Disarming the Arms Race: Outmanoeuvring Bacteria with the Art of β-Lactamase Inhibition

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Abstract: Particularly in relation to β-lactam medications, the developing problem of antibiotic resistance gravely compromises public health. In order to evade these drugs, complex systems created by bacteria manufacture β-lactamases, enzymes inactivating β-lactam antibiotics. These enzymes come into two groups: metallo-β-lactamases (MBLs) and serine β-lactamases (SBLs) depending on differing mechanisms of action and susceptibility to inhibitors. Among medicines that could affect SBLs include clavulanic acid, tazobactam, and sulbactam. MBLs are still a challenging target as they withstand conventional antibiotic. To overcome this problem, researchers are focussing on developing novel antibiotics with various modes of action and looking at new β-lactamase inhibitors-including those with an eye towards MBLs as well as SBLs. Strong antimicrobial stewardship campaigns and new medical techniques' development are only two ways to confront antibacterial resistance from numerous angles.

Keywords: β-lactam antibiotics, bacterial resistance, β-lactamases, SBLs, MBLs, antibiotic stewardship

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Introduction

Once magnificent dominion of β-lactam antibiotics, humanity's defenders against infectious diseases, is becoming evil. Years of counting on these powerful drugs, including penicillins and cephalosporins, have inadvertently produced a strong enemy: bacteria armed with a variety of resistance mechanisms (Majiduddin *et al*., 2002). Like cunning combatants, bacteria have developed skilfully to neutralise once-unquestionably strong weaponry. Some have created impenetrable barriers around themselves, therefore limiting access to the material. Others have modified the very locks on their target sites, therefore preventing the antibiotics from binding. Most unexpectedly, some have developed defences inside themselves, producing enzymes that eliminate the antibiotics before they even reach their target. This increasing resistance is a horrible truth that long shadows our ability to tackle diseases. Once-life-

saving drugs become worthless, surgeries becoming more risky, and even little illnesses can be rather severe (Jose *et al*., 2009; Chen *et al.*, 2013). Still, among this barren ground there remains a shard of hope. Scientists are fighting furiously against bacterial evolution using two major strategies. One of them is the hunt for new drugs designed to outwit opponent defence. These new combatants aim to unlock the modified locks, go past the enhanced obstacles, and stay away from the enzymatic saboteurs. This is a race against time calling for creative concepts and contemporary research. The other one is realising the continuous relevance of β-lactam, antibiotics; β- lactamase inhibitors are also under research. These shield the drugs from the enzymatic attack of the bacteria. Combining these shields with the potent medications helps us to conserve precious time while the new weapons are being development. (Draws *et al.*, 2010).

β- lactamase

Mostly present in gram-negative bacteria, β-lactamase mediate bacterial resistance to β-lactam antibiotics by means of their family of enzymes. β-lactamase dissolves the amide bond of β- lactam rings, therefore making the antibiotic worthless and producing resistance to that specific β-lactam antibiotic family. Amino acid sequence homology (Ambler *et al.,* 1991) defines the groupings of β-lactamase enzymes. Moreover, classified as groups 1 through 4 are their profiles of the substrate and inhibitor (Bush *et al.,* 1995.). Serine beta-lactamases (SBLs) Ambler A, C, and D exhibit structural similarity in their active domains. All metallo beta- lactamases, or Class B β-lactamases, are active-site zinc loaded enzymes. Category B MBLs (Bush *et al.*, 1995) are defined either by one single zinc ion or by a pair of zinc ions. All classical β-lactamase inhibitors belonging to molecular classes A or D typically inhibit group 2; group 3 MBLs are weakly inhibited by all classical β-lactamase inhibitors; group 4 penicillinases are not inhibited by clavulanic acid (Bush *et al.*, 1986; Zoe *et al.*, 2013).

Mechanisms of β-lactamases

SBLs and MBLs are the two structurally and mechanistically distinct types of β-lactamases. The majority of SBLs with clinical significance hydrolyse lactam antibiotics with nucleophilic serine. The MBLs hydrolyse β-lactam antibiotics by activating water molecules with zinc ions.

Mechanism of SBLs

SBLs class A, C, and D enzymes are broad-spectrum β-lactamases that can hydrolyse carbapenems, cephalosporins, penicillins, and aztreonam. SBLs catalyse the hydrolysis of β-lactam in two steps. by the acylation-deacylation reaction. The nucleophilic attack by Ser-70 on the carbonyl carbon of the β-lactam ring is a common hydrolytic mechanism of SBLs, following activation by either Lys-73 or Glu-166 via a water molecule producing the acyl-enzyme intermediate (Fig. 1) (Page *et al.*, 1984; Tomanicek *et al*., 2013; Vandavasi *et al.*, 2016).

Sensitive to clavulanate, sulbactam, and tazobactam inhibition, most therapeutically important class SBLs form stable covalent complexes allowing permanent binding to the enzyme (Majiduddin *et al.*, 2002; Queenan *et al.*, 2007).

Mechanism of MBLs

Zinc ions are necessary for Class B β-lactamases, or MBLs, to hydrolyse (Fig. 2) β-lactam antibiotics (Penicillins, cephalosporins, carbapenems, and cephamycins). There are no therapeutically effective inhibitors against MBLs, which are responsible for the resistance of some bacteria to antibiotics (Alistair *et al.,* 2021).

β-lactamase inhibitors

β-lactamase inhibitors are certain drugs that inhibit the SBLs and MBLs, and they are co-administered with β-lactam antibiotics to activate and reduce resistance developed against bacteria. Researchers have identified six serine-beta-lactamase (SBL) inhibitors for therapeutic use; nonetheless, there are not any drugs on the market that target the metallo-beta-lactamases

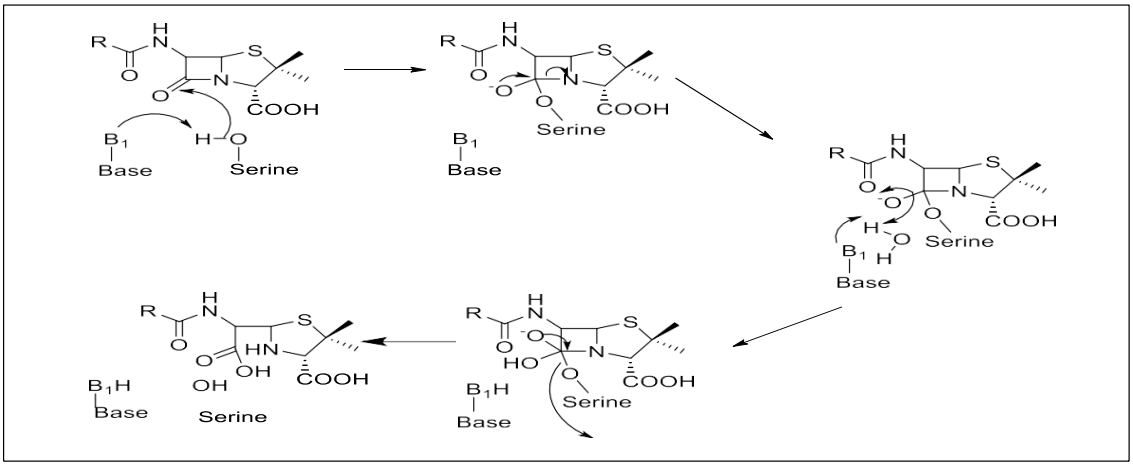

Fig. 1: Outline of the SBL hydrolysis mechanism.

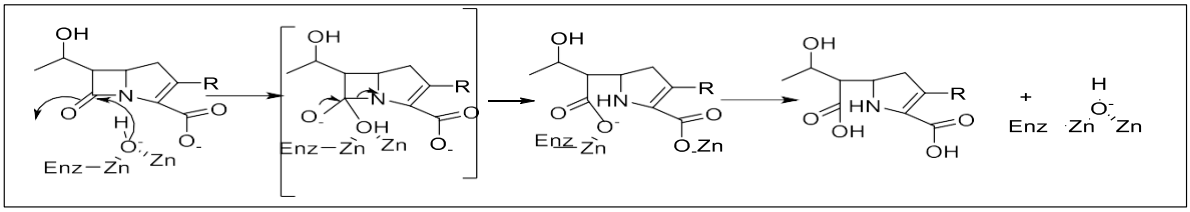

Fig. 2: Outline of the MBL hydrolysis mechanism.

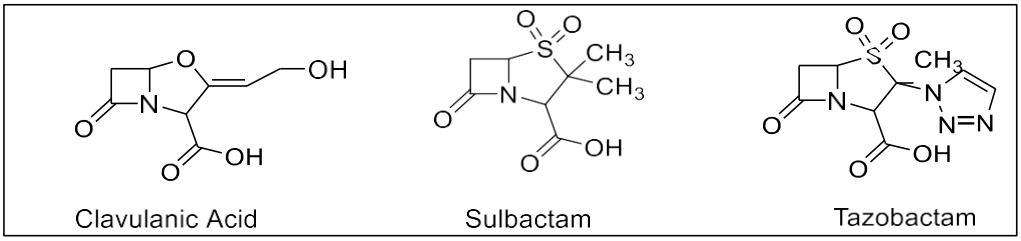

Fig. 3: Clavulanic Acid, Sulbactam, and Tazobactam.

(MBLs) or MBL-SBL dual action inhibitors. Developing strong β-lactamase inhibitors (Li *et al.,* 2022) this specific method is a fundamental tactic to raise the usage of β-lactam antibiotics. Multiple ββ-lactamase inhibitors and categorised below:

SBL Inhibitors:

Clavulanic Acid:

Clavulanic acid (Fig. 3) is biosynthesized by *Streptomyces clavuligerus* and exhibits a structural divergence from penicillin G and V characterized by an oxazolidine ring in place of a thiazolidine ring. Its mode of action entails the formation of an irreversible complex with β-lactamase in proximity to its active site, resulting in enzyme inactivation and subsequent potentiation of the antibacterial spectrum of antibiotics such as amoxicillin and ticarcillin when administered concomitantly. (Tooke *et al.*, 2019; Khanna *et al*., 2021).

Sulbactam:

One semi-synthetic β-lactamase inhibitor is

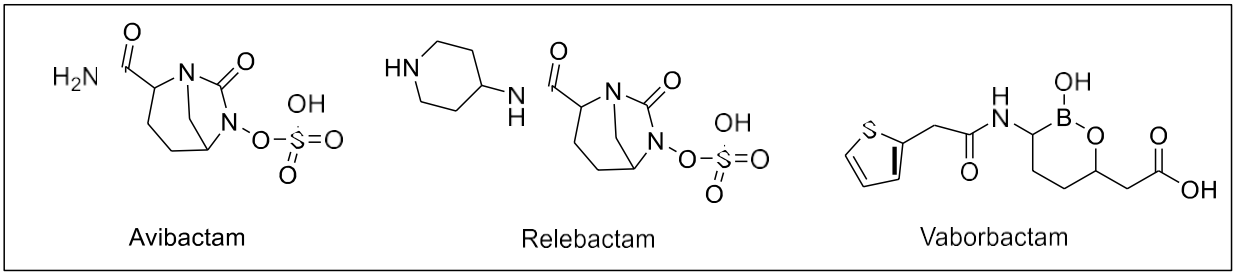

Fig. 4: Avibactam, Relebactam, and Vaborbactam.

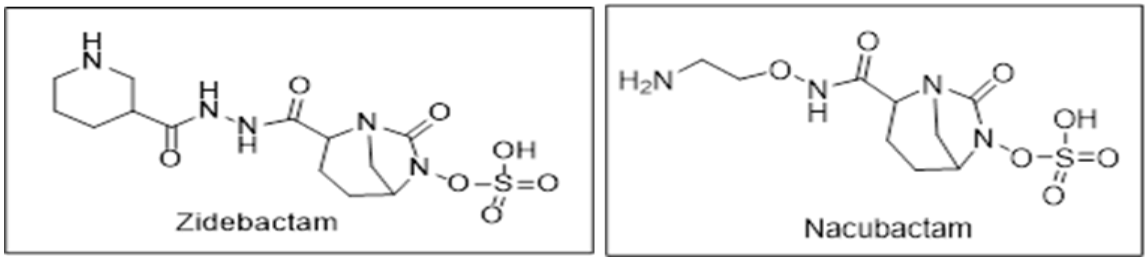

Fig. 5: Zidebactam and Nacubactam.

sulbactam (Fig. 3). Enzyme inactivation and subsequent protection of β-lactam antibiotics against hydrolysis follow from irreversible covalent bonding of its β-lactam ring with the active site serine residue of β-lactamases. Clinically, sulbactam is co-administered with antibiotics such as cefoperazone and ampicillin to enhance their antibacterial activity (Tooke *et al.*, 2019; Khanna *et al*., 2021).

Tazobactam:

Tazobactam (Fig. 3) is another β-lactamase inhibitor and a derivative of penicillanic acid. Tazobactam is differentiated from other penicillins by structural modification, which involves replacing an exocyclic methyl hydrogen with a 1,2,3-triazol-1-yl group. (Tooke *et al.*, 2019; Khanna *et al.*, 2021).

Avibactam:

Avibactam (Fig. 4), a non-β-lactam β-lactamase inhibitor co-developed by Actavis (now Teva) and AstraZeneca, is classified as an azabicycloalkane distinguished by a sulfoxy group substituting the amino hydrogen at the 6-position. Structurally divergent from other clinically employed β-lactamase inhibitors (Tooke *et al*., 2019; Khanna *et al*., 2021), avibactam lacks a β-lactam core. It is currently administered concomitantly with ceftazidime and meropenem.

Relebactam:

Relebactam (Fig. 4), a diazabicyclooctane β-lactamase inhibitor, exhibits structural similarity to avibactam with a key distinction: a piperidine ring appended to the carbamoyl group's carbon at position 2. This structural modification contributes to reduced inhibitor efflux from bacterial cells. Relebactam is currently co-administered with imipenem and cilastatin (Tooke *et al.*, 2019; Khanna *et al.*, 2021).

Vaborbactam:

Vaborbactam is a β-lactamase inhibitor composed of a cyclic boronic acid structure (Fig. 4). This unique structure allows it to strongly bind to serine β-lactamases (Tooke *et al*., 2019; Khanna *et al*., 2021). It is administered intravenously in combination with meropenem.

Zidebactam:

Showing a dual mode of action, zidebactam (Fig. 5) is a bicyclic acyl hydrazide β-lactam enhancer antibiotic. Concurrently suppressing β-lactamases (Livermore *et al*., 2017;,Tooke *et al.*, 2019; Khanna *et al*., 2021), it specifically and aggressively binds to penicillin-binding protein 2 (PBP2) in Gramme-negative bacteria. Currently under study is its therapeutic effectiveness when given parenterally with cefepime.

Nacubactam:

Nacubactam (Fig. 5) is a diazabicyclooctane-based β-lactam enhancer with a dual mechanism of action. It exhibits selective, high-affinity binding to penicillin-binding protein 2 (PBP2) in gram-negative bacteria while simultaneously inhibiting β-lactamases (Livermore *et al*., 2017; Tooke *et al.*, 2019; Mallalieu *et al*., 2020; Khanna *et al.*, 2021). Currently administered intra-venously in combination with meropenem, the full clinical efficacy of nacubactam is still under investigation.

Metallo-β-lactamase (MBL) inhibitors:

MBLs are a growing antimicrobial resistance threat, making many β-lactam antibiotics, e.g., carbapenems, cephalosporins, penicillins, and aztreonams, ineffective. All carbapenems and SBL inhibitors containing β-lactam can be hydrolysed by class B MBLs. (Walsh *et al.,* 2005). The requirement to obtain activity against a variety of pertinent enzymes with varying active site characteristics contributes to the difficulty of MBL inhibition. The progression of. MBL inhibitors have shown limited success so far in experimental setups. There are three primary clinically important MBLs, including New Delhi metallo-β-lactamase (NDM), Verona integron-encoded metallo-β-lactamase (VIM), and imipenem's (IMP) enzymes. Comprehensive reviews on certain compounds found to be preventing. MBLs have been published in a number of articles (Fast *et al*., 2005; Groundwater *et al.*, 2016; McGeary *et al*., 2017; Linciano *et al.*, 2019). There are a number of available MBL inhibitors with the functional groups thiols (Mollard *et al*., 2001; Tehrani *et al*., 2017; Buttner *et al.*, 2018) and sulfonyl hydrazones (Meng *et al*., 2019). Bis-carboxylic acids (Siemann *et al*., 2002; Feng *et al*., 2012), picolinic acids (Hiraiwa *et al*., 2014; Chen *et al*., 2017), chelating agents (Somboro *et al*., 2015; Azumah *et al*., 2016), and their bacteria-targeting analogues (Yarlagadda *et al*., 2018; Schnaars *et al.*, 2018) can bind with zinc present at the active site of the MBLs.

Aspergillomarasmine (AMA), one of several naturally occurring compounds derived from fungal extracts, has been shown to be effective *in vivo* and to be a potent inhibitor of both NDM and VIM-type enzymes. It was examined for MBL-blocking activities (Schnaars *et al.,* 2018). From streptomyces, two unique metabolites, SB 212021 and SB 212305, have been identified. These newcomers belong to the antibiotic class known as phenazine. Both compounds displayed IC50's of 1-75 microM for the metallo-beta-lactamases of Bacteroides fragilis 262 CfiA and Xanthomonas maltophilia L-1 when zinc was not added (Gilpin *et al*., 1995).

Dual Serine β-lactamases and Metallo-β-lactamases (Dual SBLs and MBLs) inhibitors:

New dual-action MBLs and SBLs inhibitors are urgently needed because the growth and spread of bacteria have already developed multidrug resistance to both metallo and serine-β-lactamase-mediated β-lactam antibiotics. (2'S) -(1-(3'-mercapto-2'-methylpropanamido) methyl) boronic acid (MS01), a novel dual action inhibitor that exhibits broad spectrum inhibition of typical MBL and SBL enzymes, was discovered through the use of a pharmacophore fusion technique (Wang *et al.*, 2019).

Conclusion

Nowadays, β-lactam antibiotics are the most highly recommended antibacterial drugs in the arsenal against infectious diseases. β-Lactam antibiotics have demonstrated their well-tolerability and excellent efficacy. Simultaneously,

resistance has been developed by bacteria with the capacity to break the peptide bond of the β-lactam ring present in the drugs. As a result, attention is being focused on creating β-lactamase inhibitors, which are given in conjunction with β-lactam antibiotics to prevent the action of enzymes that produce resistance and enhance antibiotic effectiveness. β-lactamase inhibitors are acting on SBLs and MBLs to exert their action on the microorganism, allowing the antibiotics with beta-lactams to achieve their action. From this article, we could underline some areas and a strategic approach towards reducing antibacterial resistance, and we could also show that additional research is quite justified in this field to develop dual-acting SBLs and MBLs inhibitors to generate a safe and effective antibiotic treatment environment.

Conflict of Interest

The authors declare no conflicts of interest.

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