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# Small-molecule strategies to combat antibiotic resistance: mechanisms, modifications, and contemporary approaches

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Antibiotic resistance poses a formidable threat to human health, representing a critical challenge that demands urgent attention. Without decisive feat, we confront the alarming prospect of a world where effective antibiotics are no longer available. Bacteria employ various mechanisms to elude antibiotics, including modifying antibiotic targets, utilizing efflux pumps to avoid antibiotics, and inactivating antibiotics. This review focuses on small-molecule-based approaches to overcoming resistance, with emphasis on chemical adjuvants (such as  $\beta$ -lactamase inhibitors, efflux pump inhibitors, and membrane permeabilizers), synergistic combination therapies, repurposed non-antibiotic drugs, and structural modifications of known antibiotics like ciprofloxacin. We critically analyze structure–activity relationships (SAR), biochemical mechanisms, and clinical barriers associated with each strategy. By addressing antibiotic resistance, we aim to fortify our ability to combat bacterial infections effectively and sustain the efficacy of existing antibiotics in the face of evolving resistance.

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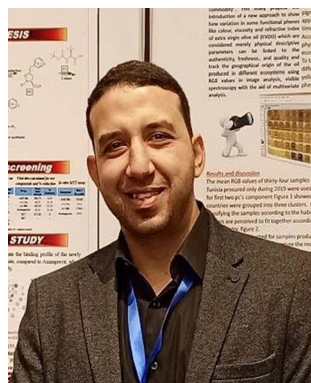
## 1. Introduction

Antibiotics have been widely recognized as crucial medications in combating infectious diseases for the last century. Paul Ehrlich, a pioneer in modern chemotherapy, is credited with

discovering the first antibiotic, Salvarsan, in 1909. This groundbreaking synthetic antibiotic, derived from arsenic, was utilized for treating syphilis, caused by *Treponema pallidum*.<sup>1,2</sup> In 1928, Alexander Fleming fortuitously discovered that the fungus *Penicillium notatum* could inhibit the growth of *Staphylococcus aureus* colonies. In 1930, Gerhard Domagk discovered the sulfa drugs.<sup>3</sup> Following World War II, semi-synthetic antibiotics like amoxicillin and quinolones were generated to improve stability and broaden antibacterial effectiveness. Macrolides, third-generation cephalosporins, and linezolid are

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among the most recent developments, which are designed to improve the pharmacokinetics of antibiotics and combat Gram-negative bacterial resistance.<sup>4,5</sup> Notwithstanding these advancements, the pervasiveness of antibiotic-resistant bacterial strains has escalated in current eras and necessitating a reevaluation of antibiotic utilization.<sup>6,7</sup>

Antibiotic resistance denotes to bacteria's capability to withstand the impact of antibiotic agents, categorized as natural or acquired resistance. Natural resistance implies inherent resistance within bacteria. Acquired resistance transpires when a bacterium develops resistance to an antimicrobial agent to which it was before susceptible. Antibiotic resistance is often acquired and may arise *via* gene changes during normal physiological processes, modifications in cellular structures, the gaining of exogenous resistance genes, or a blend of these tactics.<sup>8</sup>

In 1945, Alexander Fleming issued a public admonition against the perils of antibiotic misuse, acknowledging the hazards linked to its improper application. Excessive or unwarranted use of antibiotics might result in bacterial adaptations that render the medicines ineffective against them.<sup>9</sup> This review focuses specifically on small-molecule strategies to combat bacterial resistance. We provide a chemistry-driven perspective on the mechanisms of resistance and explore the medicinal chemistry innovations aimed at restoring antibiotic efficacy. Strategies covered include the development of adjuvants, structural modifications of existing drugs, rational combinations, drug repurposing, and novel chemical scaffolds.

## 2. Key mechanisms underlying antibiotic resistance

Knowing the processes that cause antibiotic resistance is a crucial step in finding a solution to this tricky problem. Various parts of the mechanisms of antimicrobial resistance have been reviewed in pertinent literature. We will go over the



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mechanisms that have been found in human infections that have been isolated from clinical settings. Typically, these strategies may be grouped into four main types: changing the drug's target, activating the drug efflux pump, inhibiting the absorption of the drug, and inactivating by enzymes.

### 2.1. Alteration of drug target

An explicit illustration of this process is the resistance to  $\beta$ -lactam drugs noted in Gram-positive bacteria. These bacteria modify the configuration of penicillin-binding proteins (PBPs). PBPs are transpeptidases that play a crucial role in the synthesis of peptidoglycan within the cell wall. Structural modifications of PBPs, such as PBP2a in *S. aureus* due to the acquisition of the *mecA* gene, will diminish or entirely obstruct the drug's binding capability.<sup>10,11</sup>

Additionally, certain Gram-positive bacteria, including enterococci and staphylococci, exhibit resistance to glycopeptides like vancomycin, which functions as a cell wall synthesis inhibitor. Resistance emerges *via* the acquisition of van genes, resulting in structural modifications of peptidoglycan precursors that reduce vancomycin binding efficacy.<sup>10,12</sup> vancomycin-susceptible staphylococci produce cell-wall precursors that terminate in D-Ala-D-Ala. Subsequent to their transfer from the cytoplasm to the cell membrane, these precursors exhibit a high affinity for vancomycin; upon binding, they are rendered incapable of participating in cell-wall synthesis. Vancomycin-resistant staphylococci, when exposed to an inducer such as vancomycin, produce substrates with varying termini (D-Ala-D-Lac, or D-Ala-D-Ser) that exhibit low affinity for vancomycin, enabling continued synthesis of the cell wall.<sup>13</sup>

### 2.2. Activation of drug efflux pump

Antibiotic efflux is a primary method by which bacteria eject antibiotics from their cytoplasm to the exterior environs utilizing specialized transporter proteins known as efflux pumps.<sup>14</sup> These pumps afford a protective function by extruding the antibiotic from the bacterial cell.<sup>15,16</sup> Certain entities are selective, expelling just certain substrates, while others are non-selective, accommodating a broad spectrum of structurally varied substances, including colors, organic solvents, detergents, and many families of antibiotics.<sup>17</sup> Efflux pump genes are encoded in the bacterial chromosome. Some forms of expression are constitutive, whilst others are induced or overexpressed in reaction to specific environmental stimuli or the availability of a proper substrate.<sup>18</sup>

The classification of bacterial multidrug efflux systems is based on their construction and energy source, dividing them into five primary families: resistance nodulation cell division (RND), small multidrug resistance (SMR), major facilitators (MFs), multidrug and toxic compound extrusion (MATE), and ATP-binding cassette (ABC).<sup>19</sup>

### 2.3. Inhibition of drug uptake

Bacteria have evolved methods to inhibit the antibiotic from accessing its intracellular target by reducing the absorption of the antimicrobial compound. This process is especially significant in Gram-negative bacteria because their outer membrane



consists of glycolipids, primarily lipopolysaccharide (LPS). This membrane decreases permeability and serves as a barrier to many antimicrobial drugs.<sup>20</sup>

Hydrophilic molecules, including  $\beta$ -lactams and definite fluoroquinolones, are significantly influenced by alterations in the penetrability of the outer membrane, as they typically utilize water-filled diffusion ducts, referred to as porins, to traverse this fence.<sup>21</sup> The efficiency of this natural fence is exemplified by vancomycin which is ineffective against Gram-negative germs due to its inability to invade the outer membrane. Likewise, *Pseudomonas* naturally shows lower sensitivity to  $\beta$ -lactams compared to *Enterobacteriaceae*, which is partly due to a decreased quantity and/or different patterns of porin expression.<sup>22</sup>

Numerous porin classes have been recognized and may be categorized based on their construction (trimeric or monomeric), selectivity, and regulatory mechanisms of expression. The three extensively studied porins generated by *E. coli*, specifically OmpF, OmpC, and PhoE, in conjunction with *P. aeruginosa*'s OprD (protein D2), exemplify porin-mediated antibiotic resistance. Porin modifications can transpire *via* three principal mechanisms: (i) a change in the kind of porins expressed, (ii) an adjustment in the degree of porin expression, and (iii) a disruption of porin functionality. Alterations in permeability *via* these pathways frequently lead to minimal resistance and are generally linked with additional resistance mechanisms, including heightened expression of efflux pumps.<sup>23</sup>

## 2.4. Inactivation of drug by enzymes

The majority of antibiotics work by attaching themselves to their targets in a certain way, which stops these targets' physiological functions. Nevertheless, due to prolonged struggle with antibiotics, several bacteria have developed resistance enzymes that render clinically significant drugs like aminoglycosides, carbapenems, and  $\beta$ -lactams inactive.<sup>24</sup> These resistance enzymes are primarily classified into two categories: modifying enzymes and hydrolytic enzymes.<sup>25</sup>

**2.4.1. Inactivation by hydrolytic enzymes.**  $\beta$ -Lactamases represent a substantial class of hydrolytic enzymes functioning as key agents in neutralizing  $\beta$ -lactam antibiotics.<sup>26</sup>  $\beta$ -Lactamases neutralize  $\beta$ -lactam antibiotics by hydrolyzing a definite bond within the  $\beta$ -lactam ring, leading to its structural breakdown. This structural alteration prevents the resulting compounds from binding to their target PBP proteins, ultimately rendering the antibiotics ineffective in exerting their intended therapeutic effect.<sup>27,28</sup>

Two classes of  $\beta$ -lactamases can be recognized grounded on their structure and mechanism: serine- $\beta$ -lactamases (SBLs) and metallo- $\beta$ -lactamases (MBLs). The SBLs employ a nucleophilic serine residue for the process of hydrolysis, which holds significant clinical relevance. The MBLs utilize zinc ions to trigger water molecules for the hydrolysis of  $\beta$ -lactam antibiotics, garnering significant attention in recent years due to their

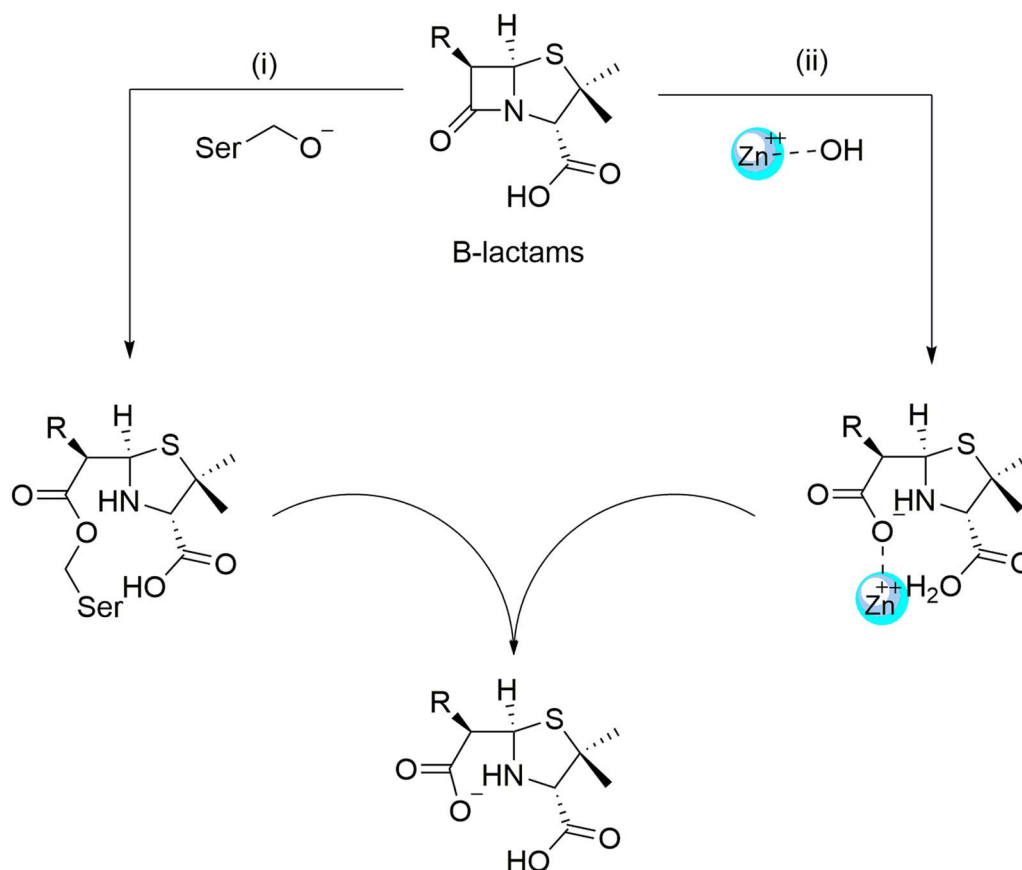


Fig. 1  $\beta$ -Lactams are hydrolyzed by (i) SBLs and (ii) MBLs.



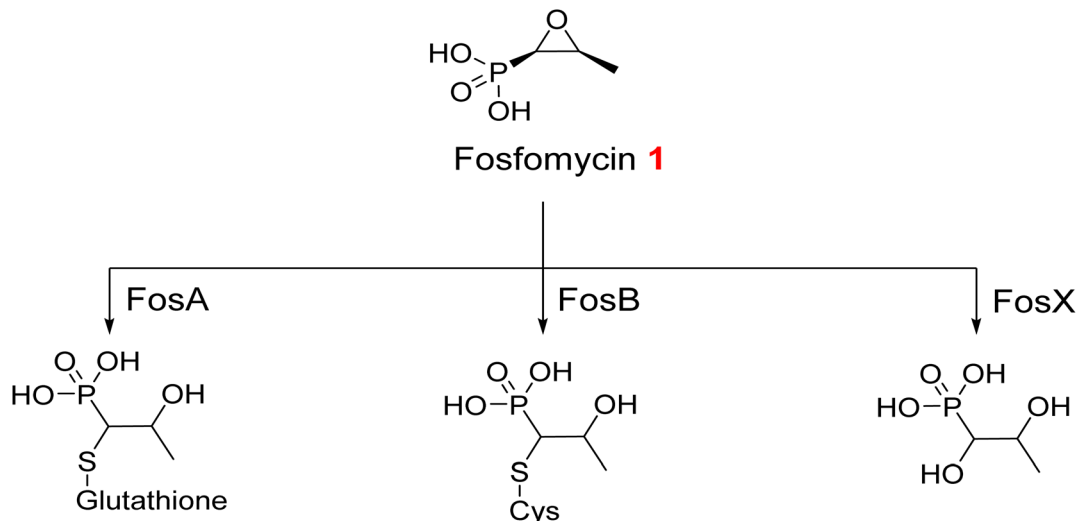


Fig. 2 Enzymatic ring-opening of fosfomycin **1** mediated by FosA, FosB, and FosX.

exceptionally broad substrate range and robust carbapenemase activity (Fig. 1).<sup>29,30</sup>

MBLs can be categorized into three distinct subclasses (B1, B2, and B3), which are primarily discriminated by their metal composition and the unique characteristics of their active sites. The majority of metallo- $\beta$ -lactamases identified to date are classified within subclass B1, with the imipenemase (IMP), Verona imipenemase (VIM), and New Delhi metallo- $\beta$ -lactamase (NDM) families being the three most prevalent metallo- $\beta$ -lactamases observed in clinical isolates.<sup>31–34</sup>

Macrolides rely on a pivotal cyclization process mediated by ester bonds to achieve their antibacterial efficacy. However, macrolide esterases possess the remarkable ability to disrupt this cyclic framework,<sup>35</sup> cleaving the ring and initiating a sequence of internal cyclization and dehydration through intramolecular condensation.<sup>36,37</sup> Accordingly, the open-ring macrolides are rendered inactive, forfeiting their antimicrobial properties.

Enzymatic hydrolysis of fosfomycin **1**, an epoxide antibiotic, transpires *via* the decomposition of its reactive epoxide ring. This process is arbitrated either by a thiol-containing co-substrate or through water-induced ring breakdown, orchestrated by three discrete fosfomycin-resistance enzymes: FosA, FosB, and FosX (Fig. 2). FosA, found in Gram-negative bacteria, functions as a glutathione transferase that depends on  $Mn^{2+}$  and  $K^+$  ions. FosB acts as an *L*-cysteine thioltransferase

requiring  $Mg^{2+}$ , whereas FosX is a  $Mn^{2+}$ -dependent epoxide hydrolase that specifically targets fosfomycin.<sup>38,39</sup>

#### 2.4.2. Inactivation by modifying enzymes

**2.4.2.1. Modification on antibiotics.** Aminoglycoside antibiotics have three basic classes of aminoglycoside-modifying enzymes: nucleotidyltransferases (ANTs), phosphotransferases (APHs), and acetyltransferases (AACs).<sup>40</sup> In addition, chloramphenicol **2** is susceptible to modification by acetyl transferase (Fig. 3).<sup>41</sup>

**2.4.2.2. Modification of antibiotic targets.** Alterations in the conformation and/or extent of penicillin-binding proteins (PBPs) represent one of the key bacterial strategies to resist  $\beta$ -lactam antibiotics.<sup>42</sup> Variations in the number of PBPs affect the amount of medication that can bind to the target.<sup>43</sup> The erythromycin ribosome methylase (*erm*) gene family methylates 16S ribosomal ribonucleic acid (rRNA), changing the drug-binding site and stopping the binding of macrolides and lincosamines.<sup>44</sup>

### 3. Strategies to tackle the problem of antibiotic resistance

#### 3.1. Use of antibiotic adjuvants

Antibiotic adjuvants are substances that exhibit minimal or no antimicrobial activity independently. They act harmonically with antibiotics to reduce or inhibit bacterial resistance, thereby

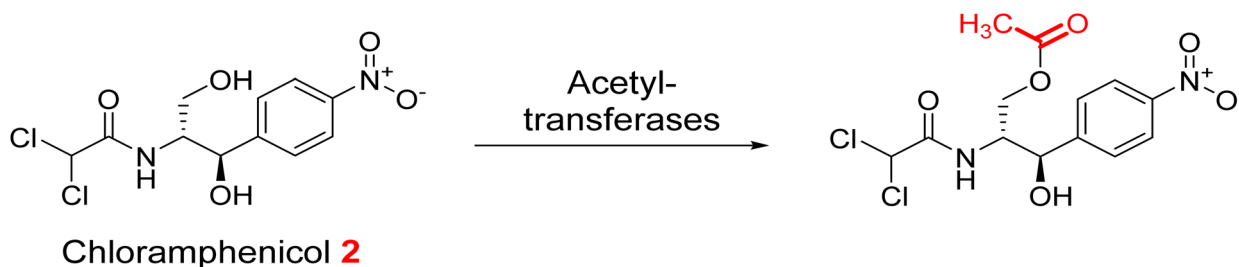


Fig. 3 Acetylation of chlormamphenicol **2**.



restoring and preserving the efficacy of antibiotics. Three primary categories of adjuvants have been investigated:  $\beta$ -lactamase inhibitors, efflux pump inhibitors (EPIs), and membrane permeabilizers.

**3.1.1.  $\beta$ -Lactamase inhibitors.** As we mentioned before, represents a substantial mechanism through which bacteria

acquire resistance to  $\beta$ -lactam antibiotics.<sup>27</sup>  $\beta$ -Lactamases can be divided into two main groups, based on their hydrolysis mechanisms: serine  $\beta$ -lactamases (SBLs) and metallo- $\beta$ -lactamases (MBLs). Therefore, a unique strategy to fight  $\beta$ -lactamase-induced resistance is the development of small-molecules

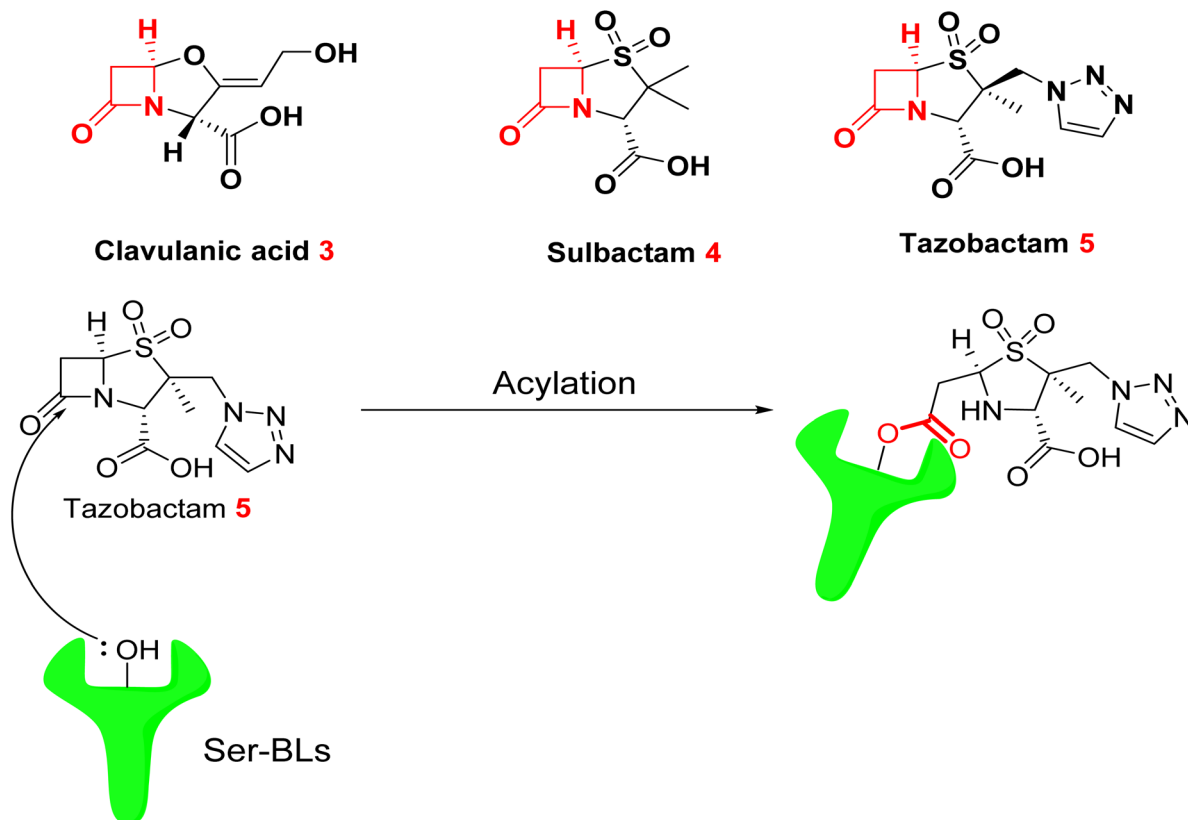


Fig. 4 Acylation of serine- $\beta$ -lactamases (SBLs) by tazobactam 5 resulting in the formation of an ester bond.<sup>49</sup>

Table 1 Comparative overview of DBO-based  $\beta$ -lactamase inhibitors

Feature	Avibactam	Relebactam	Durlobactam	Nacubactam
Chemical structure				
Core structure	Diazabicyclooctane (DBO)	DBO derivative with piperidine tail	Rigidified DBO (3,4-double bond + methyl)	DBO with PBP2 inhibition
Target enzymes	Class A, class C, some class D $\beta$ -lactamases	Class A & C $\beta$ -lactamases	Class A, C, D carbapenemases	Class A, C, some D + PBP2
Co-administered $\beta$ -lactams	Ceftazidime	Imipenem-cilastatin	Sulbactam	Meropenem, Aztreonam, piperacillin
FDA status	Approved (2014) <sup>51</sup>	Approved (2019) <sup>52-54</sup>	Approved (2023) <sup>55</sup>	Phase III trials <sup>56-58</sup>
Key features/advantages	Reversible acylation; avoids $\beta$ -lactam ring resistance	Used for pyelonephritis and complicated intra-abdominal infections	Dual inhibition; effective vs. MDR Acinetobacter	Dual action; improves antibacterial activity



inhibiting the above-mentioned  $\beta$ -lactamase enzymes and can be classified to SBLs inhibitors and MBLs inhibitors.<sup>45</sup>

### 3.1.2. Serine $\beta$ -lactamase (SBLs) inhibitors

**3.1.2.1. Classical SBLs inhibitors.** Clavulanic acid **3**, the first  $\beta$ -lactamase inhibitor approved for therapeutic utilization, was insulated from *Streptomyces clavuligerus* in the early seventies.<sup>46</sup> Sulbactam **4** and Tazobactam **5** are penicillinate sulfones that were developed later by drug developers in 1978 and 1980, respectively.<sup>47</sup> These three approved  $\beta$ -lactam-ring containing  $\beta$ -lactamase inhibitors can irreversibly acylate  $\beta$ -lactamases by targeting the enzyme's serine residue (Fig. 4)<sup>48</sup> meaning that serine- $\beta$ -lactamases are unable to hydrolyze the  $\beta$ -lactam antibiotics that are given with them (SBLs). Nonetheless, these inhibitors possess the same  $\beta$ -lactam core structure

characteristic of  $\beta$ -lactam antibiotics. Bacteria swiftly develop resistance to these structurally similar compounds by utilizing or altering pre-existing mechanisms.<sup>27</sup>

**3.1.2.2. Diazabicyclooctanes (DBOs) based SBL inhibitor.** Second-generation  $\beta$ -lactamase inhibitors with diazabicyclooctane (DBO) scaffolds have shown significant promise in restoring the activity of  $\beta$ -lactam antibiotics against resistant Gram-negative bacteria. These compounds overcome limitations of traditional inhibitors by targeting a broader range of  $\beta$ -lactamases, including Class A, C, and some D serine  $\beta$ -lactamases. This section summarizes four key DBO-based inhibitors including Avibactam **6**, Relebactam **7**, Durlobactam **8**, and Nacubactam **9** highlighting their structures, co-administered antibiotics, mechanisms, and clinical

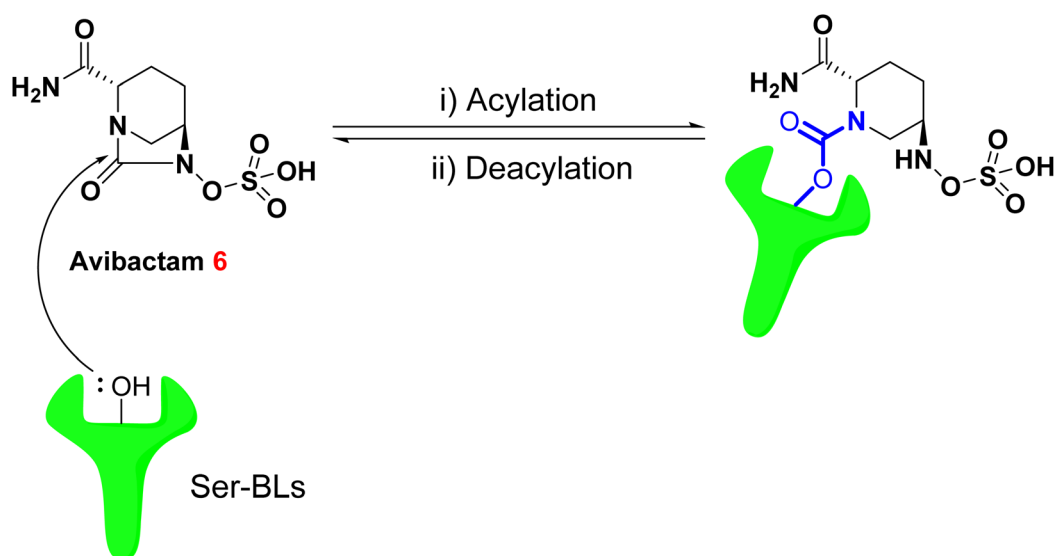


Fig. 5 Acylation of SBLs by avibactam **6** to form a carbamoyl linkage.<sup>49</sup>

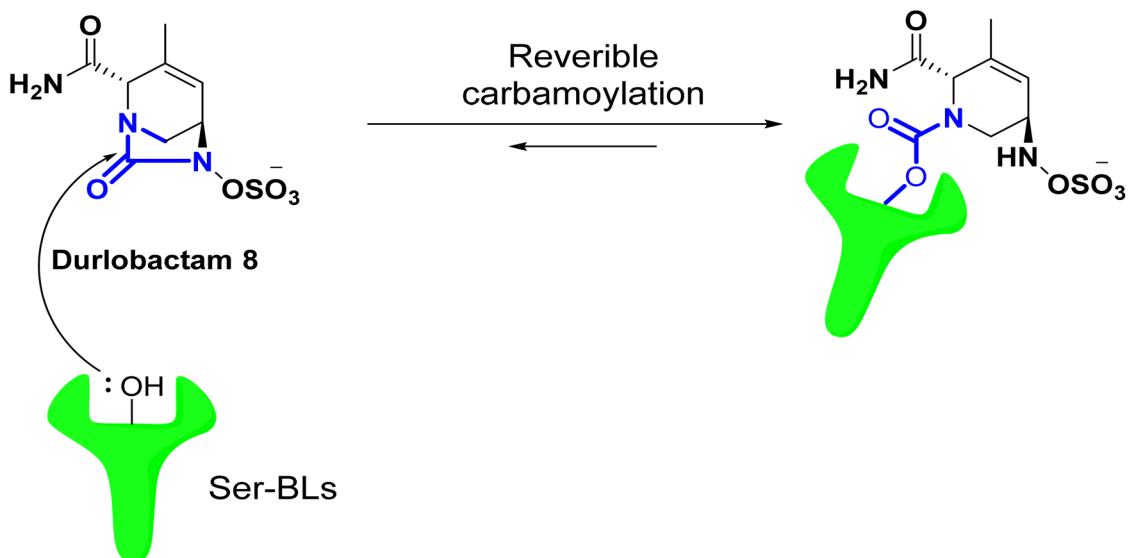


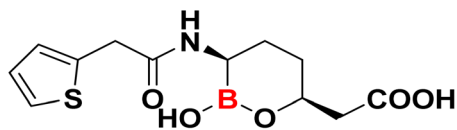
Fig. 6 Mechanism of action of durlobactam **8**.



relevance.<sup>50</sup> Table 1 below presents a comparative analysis between the four DBO inhibitors, whereas Fig. 5 and 6 depict the structural and mechanistic attributes of Avibactam and Durlobactam, respectively as a representative examples of DBO-based  $\beta$ -lactamase inhibitors.

### 3.1.2.3. Boronate $\beta$ -lactamase inhibitors

3.1.2.3.1. *Varborbactam*. Varborbactam **10** represents the inaugural non- $\beta$ -lactam boronic acid  $\beta$ -lactamase inhibitor. In



Varborbactam **10**

2017, the FDA confirmed a mixture of meropenem and vaborbactam to manage complicated urinary tract infections in adult patients.<sup>59,60</sup> Varborbactam demonstrated a diverse array of effectiveness against several serine  $\beta$ -lactamases, encompassing class A carbapenemases and class C cephalosporinases. However, it exhibited minimal activity against class B metallo- $\beta$ -lactamases and class D carbapenemases.<sup>61,62</sup> The combination of meropenem and vaborbactam safeguards meropenem from being degraded by serine carbapenemases through a novel enzyme inhibition mechanism. This process involves vaborbactam binding covalently to the catalytic serine residue within  $\beta$ -lactamase enzymes. The boron atom in vaborbactam mimics the tetrahedral intermediate that naturally forms during the enzyme's acylation and deacylation steps in  $\beta$ -lactam hydrolysis. By stabilizing this transition state analogue, vaborbactam effectively blocks the enzyme's activity, leading to rapid and irreversible inactivation of  $\beta$ -lactamases, thereby protecting meropenem from degradation.<sup>63,64</sup>

3.1.3. **Metallo- $\beta$ -lactamases (MBLs) inhibitors.** The creation of an effective metallo- $\beta$ -lactamase inhibitor is a formidable challenge for medicinal chemists for many reasons: (1) distinct mechanisms of catalysis: metallo- $\beta$ -lactamases and serine  $\beta$ -lactamases operate through fundamentally distinct catalytic mechanisms. As a result, existing serine  $\beta$ -lactamase inhibitors are ineffective against MBLs. (2) Variability in active sites: the essential characteristics of the active pockets differ greatly between the two enzyme classes. While serine  $\beta$ -lactamases have a deep and narrow catalytic active pocket, MBLs feature a shallow groove with limited binding contacts for inhibitors or substrates. (3) Structural and functional diversity: MBLs exhibit significant structural diversity, including low homology across active site residues and variability in  $Zn^{2+}$  concentration, which complicates the design of universal inhibitors.

(4) Biological versatility: in contrast to serine  $\beta$ -lactamases, which are exclusively bacterial enzymes, metallo- $\beta$ -lactamases (MBLs) are part of a more extensive superfamily of metalloproteins that engage in a variety of biological functions that extend beyond the hydrolysis of  $\beta$ -lactams.<sup>65</sup> Consequently, there are now no effective MBL inhibitors utilized in clinical

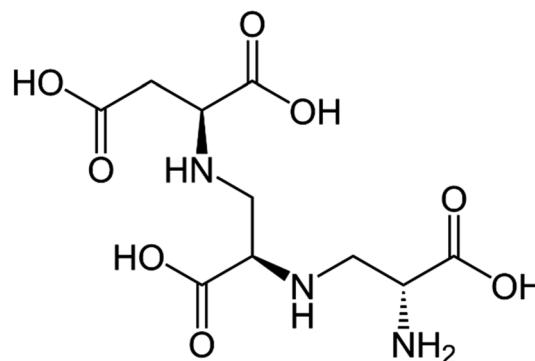
practice. The challenges encountered have hindered the advancement of clinically approved MBL inhibitors, highlighting the necessity for novel strategies in inhibitor design.

MBLs are categorized into three subclasses: B1, B2, and B3, based on the quantity of zinc atoms present. The B1 subtype is the most clinically significant and encompasses New Delhi. Metallo- $\beta$ -lactamase-1 (NDM-1) is an enzyme that poses a significant hazard to human health due to its substrate



promiscuity, broad-spectrum activity, emergence of variations, and capacity for transferability. The pursuit of an effective NDM-1 inhibitor continues, and despite extensive research over the years, an optimal treatment remains elusive. Significant advancements in NDM-1 research are underway, with several molecular structures having been altered and evaluated against NDM-1. In the past decade, a substantial volume of research on NDM-1 inhibitors has been published.

3.1.3.1. *Natural MBLs inhibitors.* Aspergillomarasamine A (AMA) **11** is a polyamino acid synthesized by *Aspergillus versicolor* that effectively inhibits antibiotic resistance enzymes in Gram-negative pathogenic bacteria, including *Pseudomonas* spp, *Acinetobacter* spp, and *Enterobacteriaceae*.<sup>66,67</sup> Despite never achieving clinical significance as a therapy, it has been reassessed for NDM-1 inhibition. This molecule is particularly noteworthy due to its capacity to bind zinc while exhibiting benign effects *in vivo*, as demonstrated in mice. A 95% survival rate was observed post-infection with NDM-1 positive *K. pneumoniae*, but meropenem monotherapy resulted in significantly higher death.<sup>67</sup>

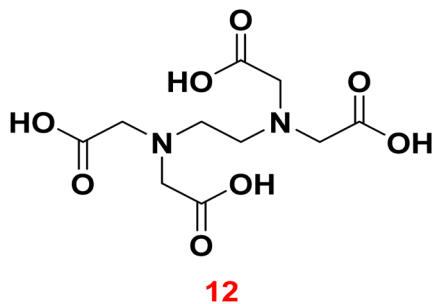


Aspergillomarasamine A (AMA) **11**

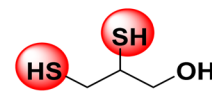
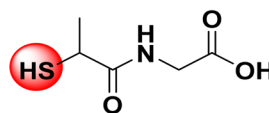
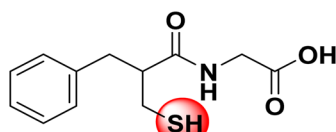
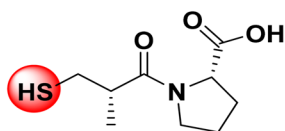
3.1.3.2. *Chelating agents.* Initial compounds recognized as NDM-1 inhibitors were chelating drugs like ethylenediamine-*N,N,N',N'*-tetracetic acid (EDTA) **12** and trispicolylamine (TPA)



13.<sup>68,69</sup> These compounds bind zinc at the active site of MBL enzymes, therefore limiting their action and facilitating the efficacy of antibiotics. Regrettably, the prevalence of metal-containing enzymes in the human system renders chelating drugs unsuitable for clinical use due to their ubiquitous cytotoxicity.



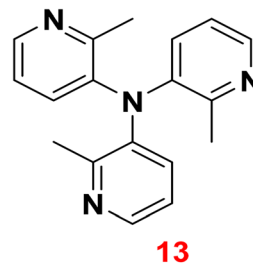
3.1.3.3. *Thiol comprising congeners.* The thiol group is renowned as a zinc chelator, making its presence in initiatives focused on discovering new inhibitors for class B  $\beta$ -lactamases unsurprising.<sup>70–72</sup> In 2015, Klingler *et al.* evaluated eleven authorized pharmaceuticals or their bioactive metabolites featuring a free thiol group, identifying four medicines that inhibit the clinically significant metallo- $\beta$ -lactamases NDM-1, Verona integron-encoded metallo- $\beta$ -lactamase (VIM-1), and imipenemase-7 (IMP-7).<sup>73</sup> These inhibitors include captopril **14**, a clinically approved angiotensin-converting enzyme (ACE) inhibitor commonly used for the treatment of hypertension; thiorphan **15**, functioning as an enkephalinase inhibitor and is used primarily as an antidiarrheal agent; tiopronin **16**, a therapeutic agent utilized in managing severe cases of homozygous cystinuria by reducing cystine levels in the urine, and dimercaprol **17**, an established chelating agent employed in the management of heavy metal toxicity. These compounds exhibited  $IC_{50}$  values in the low micromolar range for all evaluated metallo- $\beta$ -lactamases and shown significant synergy with imipenem.



The crystal structure of NDM-1 complexed with captopril reveals that the thiolate group inhabits the fourth coordination site for each ion, facilitating intercalation between the two  $Zn^{2+}$

ions. The hydroxide anion positioned between the two  $Zn^{2+}$  ions is substituted by the thiolate group, therefore initiating the hydrolytic process (Fig. 7).

3.1.3.4. *Bisthiazolidines (BTZs).* Bisthiazolidines are thiol-containing bicyclic compounds that interact with the dizinc cores of metallo- $\beta$ -lactamases (MBLs) *via* free thiol groups,



therefore substituting hydroxide ions in the dizinc clusters during hydrolysis (Fig. 8).<sup>75</sup> These compounds have two principal advantages: extensive action against all class B enzymes and effortless production. Further refinement of the bisthiazolidine framework to establish additional binding interactions with active site residues could enhance their inhibitory efficacy, potentially reaching the desired nanomolar range.

In 2015, González *et al.* elucidated the identification of bisthiazolidines **18a,b** and **19a,b** as novel metallo- $\beta$ -lactamase inhibitors capable of reinstating the efficacy of imipenem.<sup>76</sup> The compounds in question exhibit certain traits reminiscent of  $\beta$ -lactam compounds, which serve as effective substrates for these enzymes. They possess a bicyclic configuration characterized by a bridging nitrogen atom and a carboxylate group situated in the  $\alpha$ -position relative to the nitrogen atom. Furthermore, they possess the thiol group essential for coordination with the catalytic  $Zn^{2+}$  ion. Bisthiazolidines **18a,b** and **19a,b** have demonstrated remarkable versatility as inhibitors, effectively targeting a broad spectrum of class B enzymes, including NDM-

1, VIM-2, IMP-1, and BcII, all exhibiting values within the low micromolar range.<sup>75</sup>



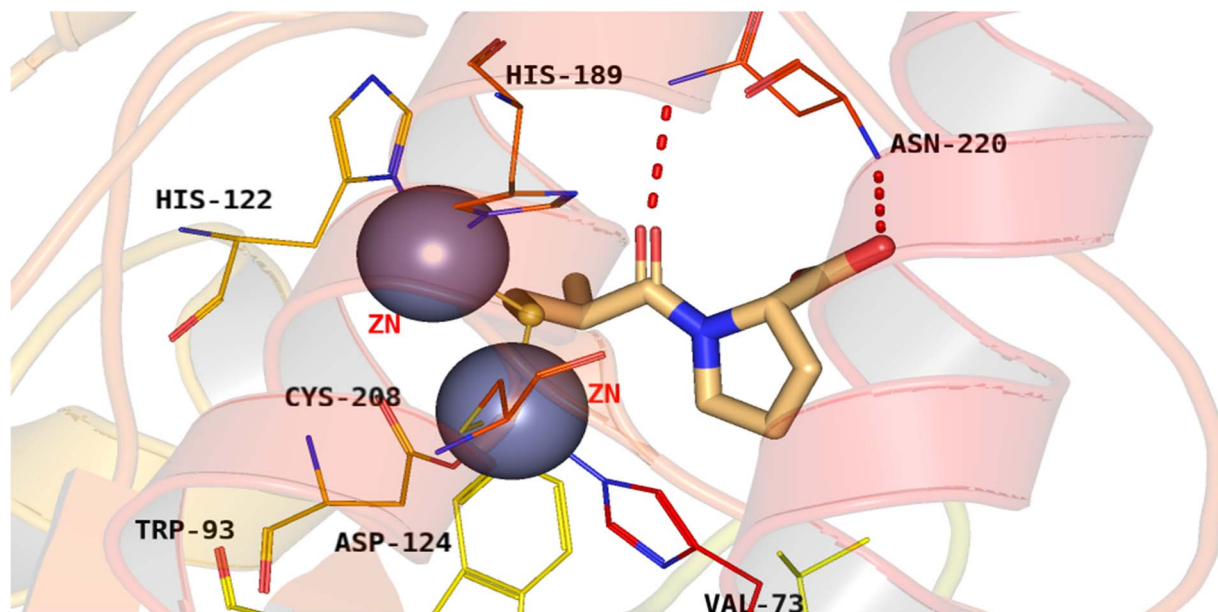


Fig. 7 Crystal structure of NDM-1 bound to captopril (PDB; 4EXS).<sup>74</sup>

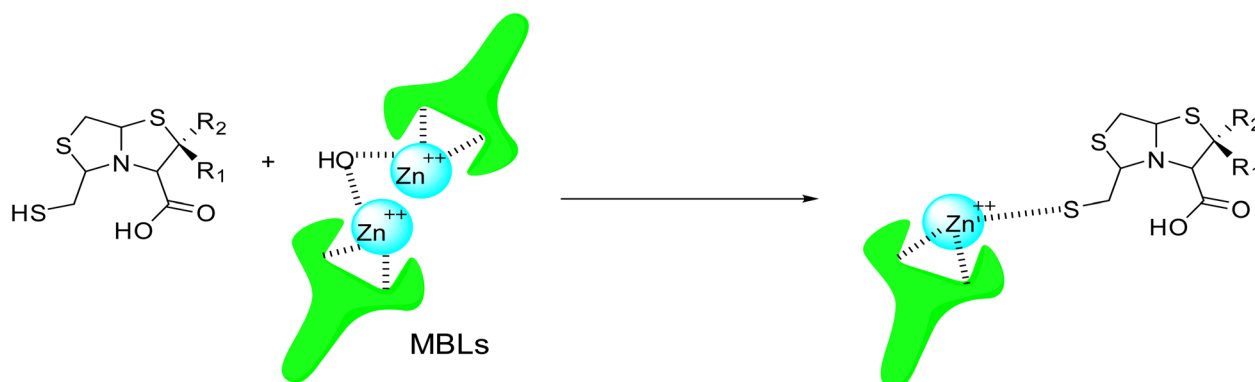
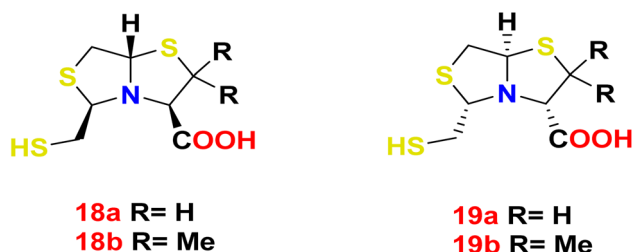
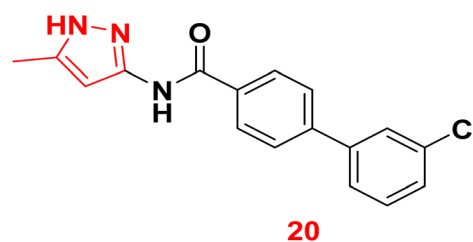


Fig. 8 Mechanism of action of bithiazolidines as MBLs inhibitors.<sup>49</sup>



**3.1.3.5. Pyrazole derivatives as NDM-1 inhibitors.** In 2021, Ahmad *et al.*, reported the design and synthesis of a series of pyrazole-based derivatives specifically developed as potent inhibitors of the NDM-1 enzyme, addressing the critical challenge of resistance posed by metallo- $\beta$ -lactamases. The *in vitro* antibacterial effect against NDM-1-positive *Acinetobacter baumannii* and *Klebsiella pneumoniae* of the target derivatives were

determined. Furthermore, a molecular docking analysis of the candidate molecules against NDM-1-producing *A. baumannii* was achieved to explore their binding interactions. Among the compounds, compound **20** exhibited the strongest binding affinity to the receptor, highlighting its potential as a remarkable antibacterial agent (Fig. 9).<sup>77</sup>



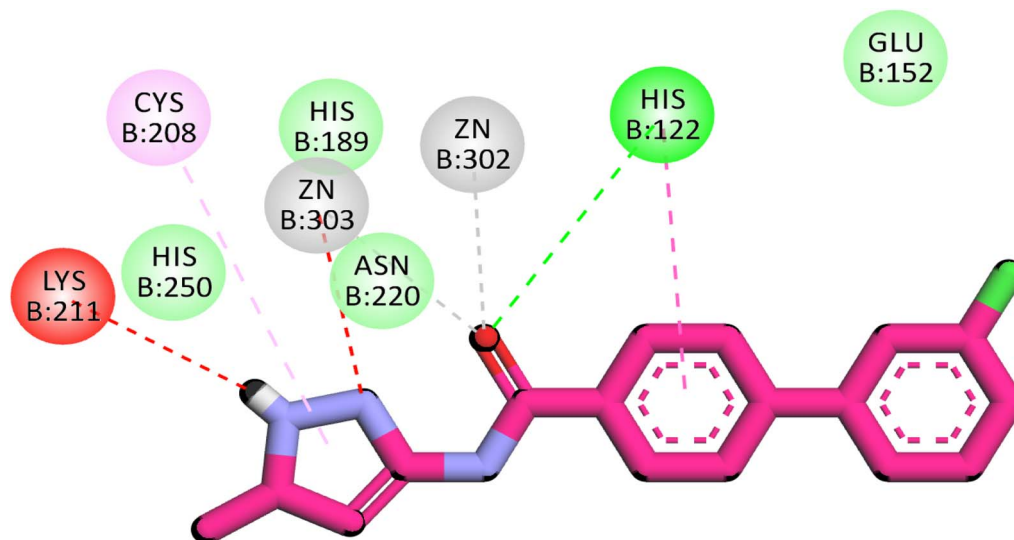


Fig. 9 Two-dimensional diagram of compound 20 with NDM1 protein (Pdb: 4EXS).

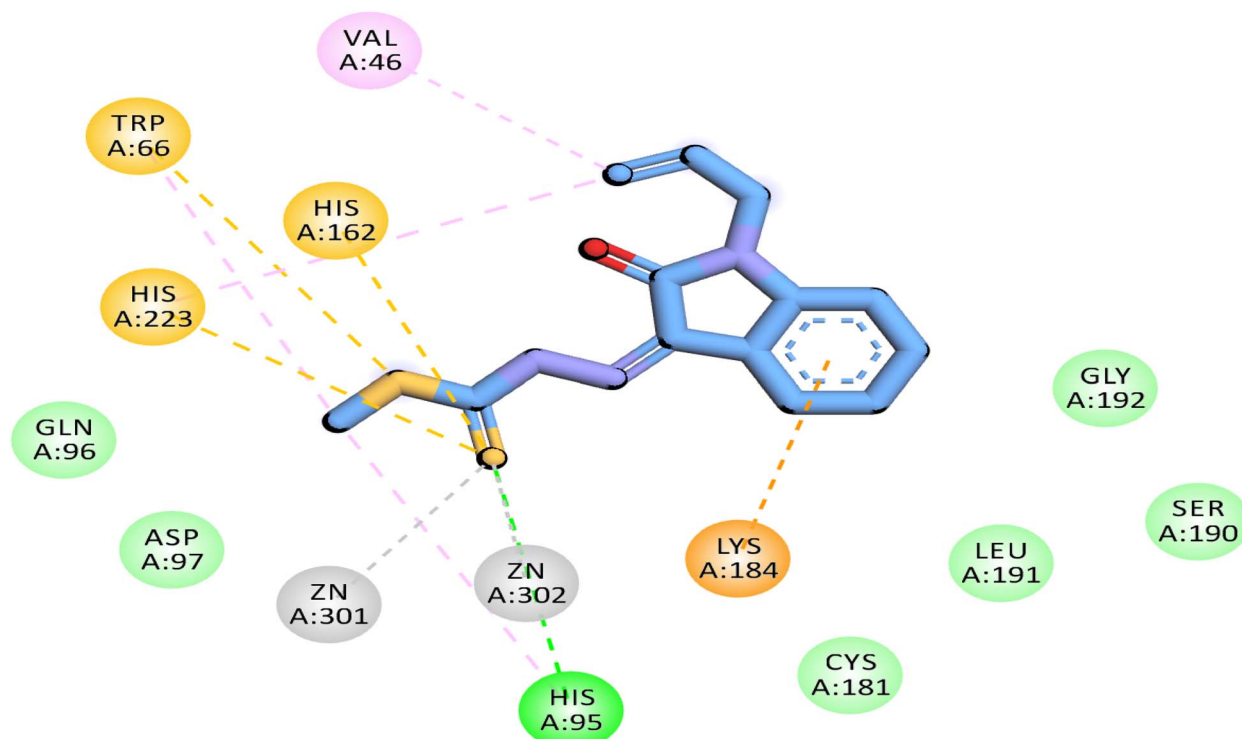
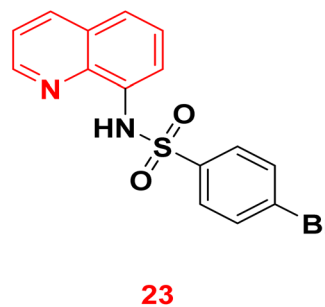
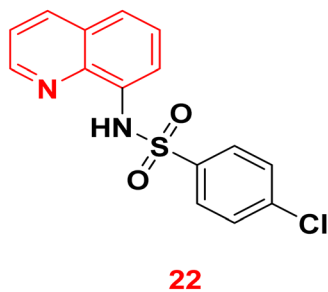
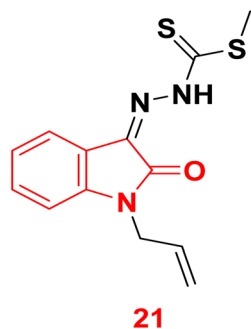


Fig. 10 Binding mode of compound 21 with NDM-1 protein (Pdb: 5ZIO).

**3.1.3.6. Isatine as NDM-1 inhibitor.** In 2023, compound 21 (Zndm19) was identified to treat drug-resistant bacterial infections through evaluation of NDM-1 enzyme activity suppression. The biological *in vitro* outcomes verified the compound 21 suppressed NDM-1 activity and reinstating the bactericidal activity of MEM against NDM-1-positive *E. coli*. The mice peritonitis infection model study revealed that the Zndm19-

meropenem combination therapy exhibited synergistic activity leading to nearly a 60% rise in survival rates along with lowered bacterial load in tissue. Furthermore, molecular docking revealed the ability of Zndm19 to chelate the two zinc centers and interact with the key amino acid residues within the active site of NDM-1 (Fig. 10).<sup>78</sup>





**3.1.3.7. Quinoline derivatives as NDM-1 inhibitors.** In 2022, thirty-one quinolinyl sulfonamides and sulfonyl esters were synthesized and evaluated for their inhibitory activity against metallo- $\beta$ -lactamase NDM-1.<sup>79</sup> Among these, compounds 22 and 23 revealed the highest inhibitory potency, with  $IC_{50}$  values of 0.02  $\mu$ M using meropenem (MEP) as the substrate. Structure–activity relationship analysis revealed that halogen substitution on the phenyl group significantly enhances the inhibitory

effects of quinoline derivatives on NDM-1. Minimum inhibitory concentration (MIC) assays showed that quinolinyl sulfonamides improved the antibacterial effectiveness of MEP against *Escherichia coli* strains expressing NDM-1 (EC01 and EC08), resulting in a 2–64-fold reduction in MICs. *In vivo* mouse studies demonstrated that compound 22 synergized with MEP, significantly reducing the bacterial load of EC08 in the liver and spleen following a one intraperitoneal dose. Molecular docking investigation showed that the endocyclic nitrogen atom of the quinoline ring and the exocyclic nitrogen of the sulfonamide group directly synchronize with the  $Zn^{2+}$  ions in the active site of NDM-1, as depicted in Fig. 11.

**3.1.4. Efflux pumps inhibitors.** Inhibition of efflux pumps is a potential approach to reinstate antibiotic sensitivity in resistant bacterial strains and avert the formation of novel resistant variants.<sup>80</sup> This can be accomplished by several means, including as suppressing the gene expression of efflux pumps, interrupting the assembly of pump components at the bacterial membrane, obstructing the membrane output duct, or depleting the energy supply necessary for pump function.<sup>81</sup> The

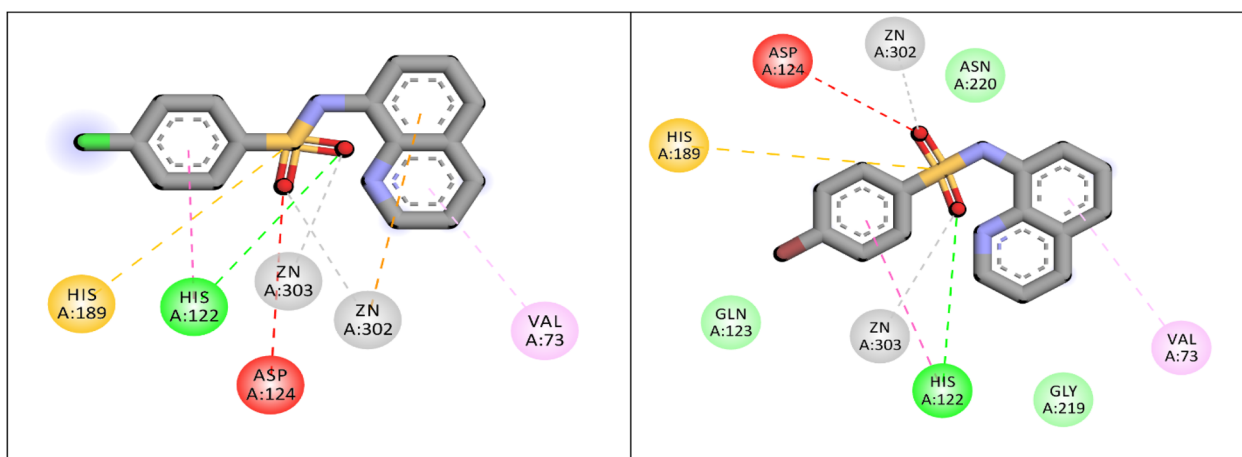


Fig. 11 Docking conformation of quinolinyl sulfonamide 22 and 23 docked into the active site of NDM-1 (PDB code 4EYL).<sup>79</sup>



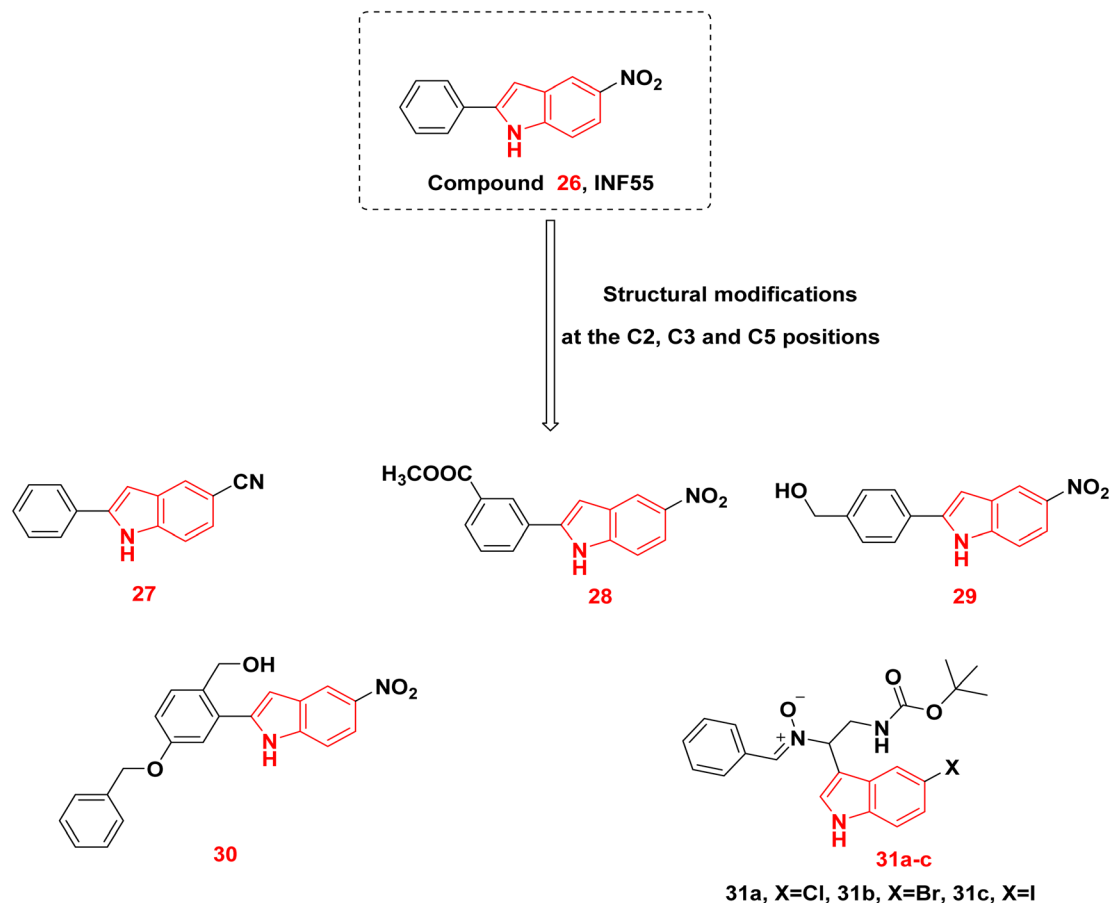
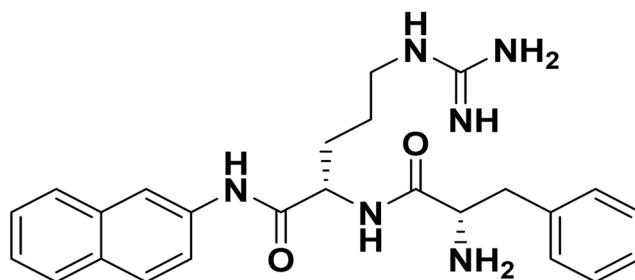


Fig. 12 Chemical structures of indole-based candidates observed to bind to NorA.

formulation of efflux pump inhibitors (EPs) necessitates meticulous evaluation of the targeted bacterial pathogen, the particular efflux pump in question, and the properties of the antibiotic to be augmented. Moreover, pharmacokinetic and pharmacodynamic characteristics are crucial to guarantee the effectiveness of EPs. An ideal inhibitor should specifically target bacterial efflux pumps while sparing eukaryotic cells, attain effective serum concentrations, and demonstrate high specificity and effectiveness, alongside a favorable therapeutic

index and pharmacokinetic profile. To mitigate the danger of resistance development, EPs must possess no intrinsic anti-bacterial action and be completely devoid of toxicity, even at elevated dosages. This holistic strategy can improve antibiotic efficacy and address the worldwide challenge of antimicrobial resistance.<sup>82</sup>

3.1.4.1. *Phenylalanine-arginine  $\beta$ -naphthylamide (PA $\beta$ N)*. It is worth highlighting that phenylalanine-arginine  $\beta$ -naphthylamide (PA $\beta$ N) 25 might have a dual antibiotic adjuvant



Phenylalanine-arginine  $\beta$ -naphthylamide (PA $\beta$ N) 25

action.<sup>83–85</sup> As it is one of the most studied EPIs through competitive inhibition mechanism, where the efflux pumps recognize it as a substrate instead of the target antibiotics (quinolones mainly ciprofloxacin and levofloxacin). Moreover, it has recently been shown to permeabilize bacterial membranes and enhanced the efficacy of  $\beta$ -lactams against overexpressing strains of *P. aeruginosa*.<sup>83</sup>

The upregulation of genes producing NorA and related efflux pumps significantly contributes to drug and biocide resistance in *S. aureus*. Consequently, NorA represents a compelling target in medicinal chemistry, since a chemical capable of inhibiting its actions might reinstate the efficacy of substrate antibiotics like fluoroquinolones. No NorA EPI has received approval for clinical application to yet.

**3.1.4.2. Indole based weapons as promising NorA efflux pump inhibitors.** The indole moiety has demonstrated potential regarding the EPI action of a drug. Compound **26** is among the initial indole-based inhibitors of NorA and can enhance the sensitivity of *S. aureus* to ciprofloxacin by four-fold.<sup>86</sup>

Subsequent structural changes were implemented at the C2, C3, and C5 locations of compound **26** (Fig. 12). In the primary identified members, an electron-withdrawing group was consistently retained (a NO<sub>2</sub> group in **28**, **29**, **30** or a CN group in **27**). Moreover, an aromatic moiety with various substituents was consistently maintained across all compounds to optimize their efficacy. Recently, indoles containing halogens at the C5 position and a distinctive nitron moiety at the C3 position (compounds **31a–c**) have garnered significant attention due to their potential as pharmacologically active agents, demonstrating promising inhibitory activity against key bacterial enzymes. All the engineered members had favorable NorA EPI characteristics, equal to or exceeding those of the lead compound **26**.<sup>87–89</sup>

Next, Lepri *et al.*, have synthesized 48 indole derivatives *via* substitutions at the C5 and N1 positions of indole.<sup>90</sup> The synthesized members have been tested against *norA*-overexpressing *S. aureus*. Compound **32** was identified as the lead and subsequently refined through modifications to four

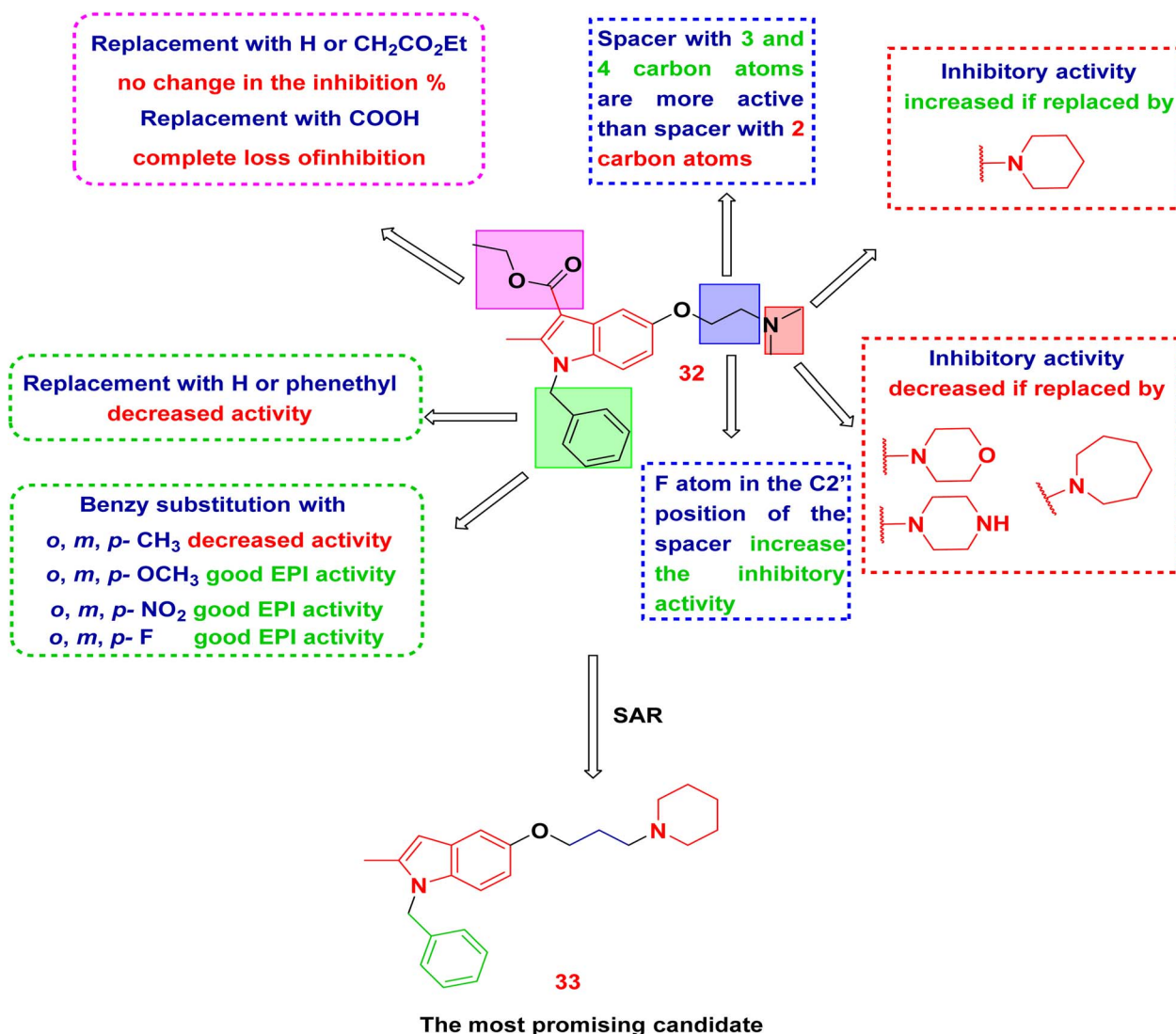


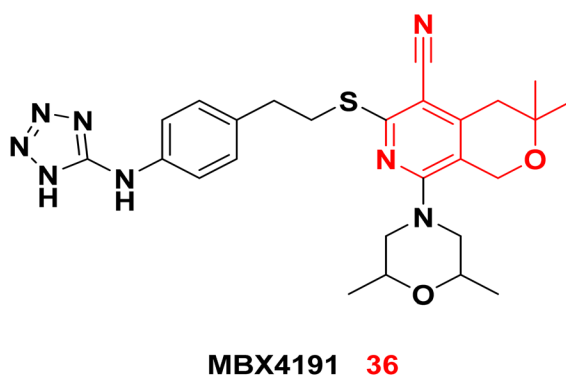
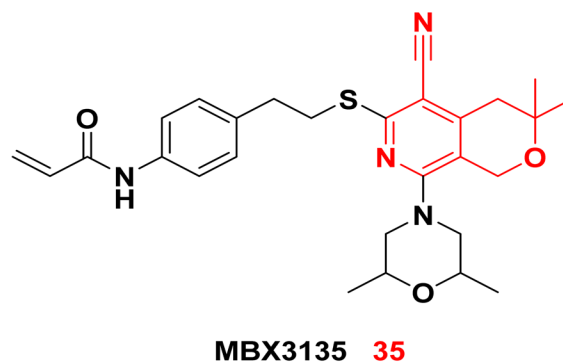
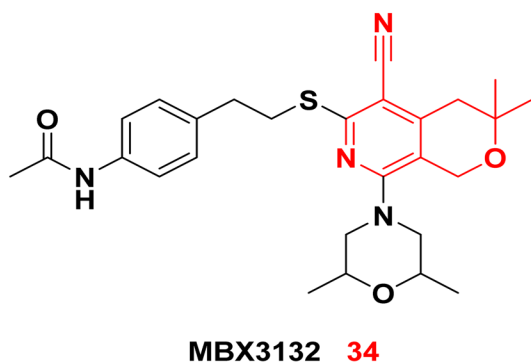
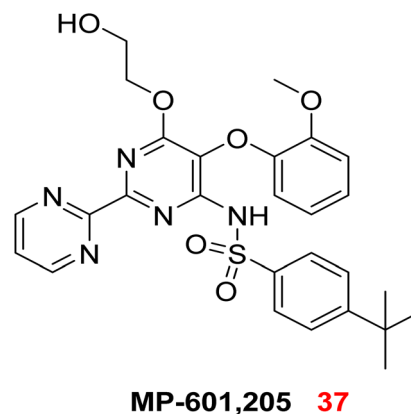
Fig. 13 Detailed structural modification of lead compound **32** and SAR study.



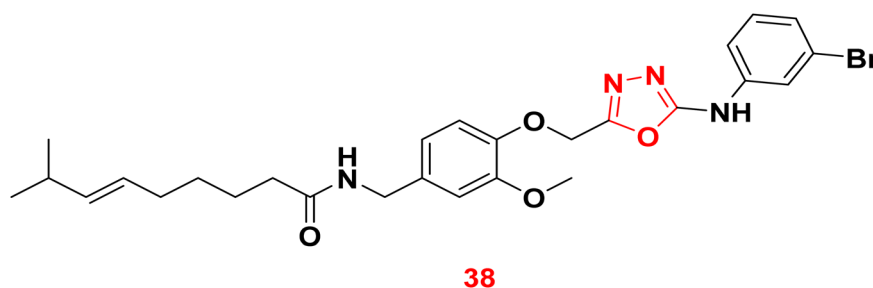
essential structural elements: the terminal dimethylamine moiety, the ethyl linker, the benzyl group, and the ethyl carboxylate (Fig. 13). Among the synthesized indole derivatives, compound **33** distinguished itself as the most formidable candidate. The analysis of the structure–activity relationship (SAR) indicated that the presence of a propoxyl chain featuring terminal cyclic amino groups is essential for the effective inhibition of the NorA pump at low micromolar concentrations. Furthermore, the *N*-benzyl moiety was observed to not only preserve inhibitory activity but also to affect biological effects and ADME properties depending on its substituents.

**3.1.4.3. Pyranopyridines as NorA efflux pump inhibitors.** Sjuts *et al.*<sup>91</sup> developed a series of pyranopyridines that demonstrated greater potency than PAβN in sensitizing Enterobacteriaceae to antibiotics. The two pyranopyridine compounds, MBX3132 **34** and MBX3135 **35**, demonstrated potency at a concentration as low as 0.1 μM, which is 500-fold lower than that of PAβN. Given the significant steric hindrance, **34** and **35** exhibited a stronger binding affinity to the binding pocket of the AcrB transporter in *E. coli* compared to PAβN. Consequently, **34** and **35** demonstrated effective inhibition at remarkably low concentrations. To improve solubility and biocompatibility, the terminal acetyl group was modified to a polar tetrazole as in MBX4191 **36**.<sup>92</sup> Compound **36** exhibited significant water solubility and minimal cytotoxic effects.

The challenge in targeting efflux pumps arises from their diverse physiological functions, which may lead to unforeseen toxicities upon inhibition. Research is thus directed towards identifying agents that specifically stop pumps working exclusively in prokaryotes.<sup>17</sup> In response to this need, numerous scientific trials have been conducted to ascertain the blockers of these pumps, as well as to explore the application of efflux pump inhibition strategies. Currently, no efflux pump suppressor have been ratified for the treatment of bacterial infections affecting humans and animals. The only reported inhibitor is MP-601, **37**, which is supplied as an aerosol in combination with ciprofloxacin for treating respiratory infections in patients with ventilator-associated pneumonia caused by a multidrug-resistant *P. aeruginosa*.<sup>73,93</sup>



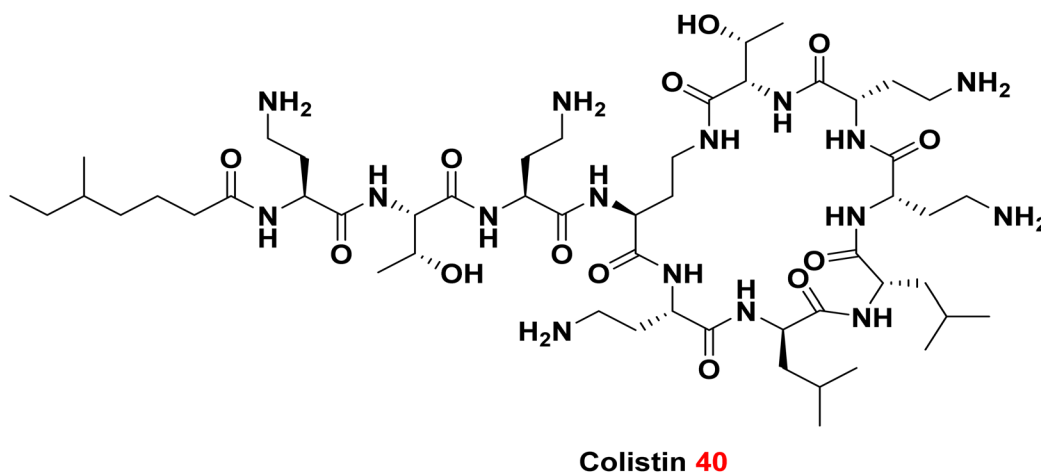
**3.1.4.4. Oxadiazoles as NorA efflux pump inhibitors.** In 2021, a new series of 1,3,4-oxadiazole conjugates linked to capsaicin was synthesized. The evaluation of ciprofloxacin activity potentiation was conducted for the entire set of synthesized members. Among them, compound **38** demonstrated significant activity. The results obtained indicated that compound **38** exhibited inhibition of the NorA efflux pump in the ethidium bromide (EtBr) fluorescence assay, significantly reducing the efflux of ethidium bromide.<sup>94</sup>

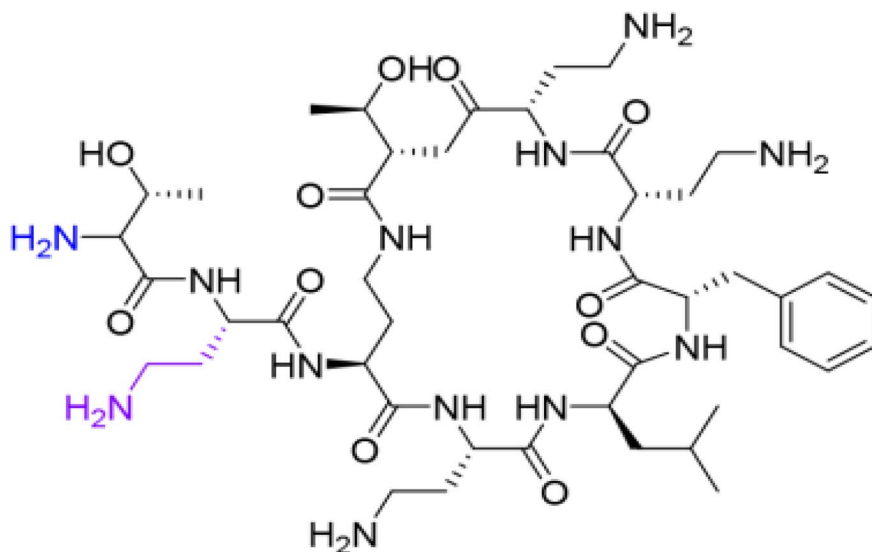


**3.1.5. Outer membrane (OM) permeabilizers.** The application of permeabilizers has been demonstrated as an effective strategy to enhance antibiotic uptake. These agents are typically cationic and amphiphilic or function as chelators, disrupting the outer membrane's structural integrity. They achieve this by either interacting with polyanionic lipopolysaccharides or sequestering outer membrane cations. This disruption increases membrane permeability, thereby facilitating drug entry. Polymyxins (*e.g.*, colistin), cationic peptides, cationic derivatives of cholic acid, and polyamine are all examples of outer membrane permeabilizers.<sup>95</sup>

**3.1.5.1. Colistin.** Colistin **40** is a naturally derived polymyxin antibiotic isolated from *Bacillus polymyxa*, composed of a decapeptide with six amino acids forming a cyclic peptide ring linked to a fatty acid side chain. It belongs to the class of antimicrobial peptides, characterized by cationic and amphiphilic properties, which have been extensively investigated as antibiotic adjuvants.<sup>96,97</sup> Colistin primarily targets the negatively charged lipid A moieties within lipopolysaccharides (LPS). Due to its stronger affinity for LPS compared to divalent cations such as  $Mg^{2+}$  and  $Ca^{2+}$ , colistin competitively displaces these ions,

destabilizing the outer membrane by releasing LPS molecules and creating permeabilized pores. The ability of colistin to form physical pores in the outer membrane enables synergistic effects with various antibiotic classes, including rifampicin and carbapenems.<sup>98,99</sup> Although colistin is an older antimicrobial agent that was previously withdrawn because of nephrotoxicity and neurotoxicity concerns,<sup>100</sup> it has re-emerged as a last-resort therapy for infections caused by carbapenem-resistant Gram-negative bacteria. Consequently, clinical administration of colistin necessitates vigilant monitoring of key biomarkers to manage potential toxicities during treatment.<sup>101</sup>





### Polymyxin B nonapeptide (PMBN) 41

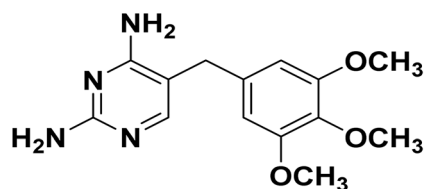
**3.1.5.2. Polymyxin B nonapeptide (PMBN).** This derivative of polymyxin-B is characterized by a shorter fatty acid lipid tail deficiency while maintaining the outer membrane-permeabilizing activity of polymyxin B.<sup>102</sup> PMBN binds to LPS, leading to the release of divalent cross-linkers that enhance outer membrane permeability. PMBN enhances the sensitivity of *E. coli* to hydrophobic antibiotics by augmenting outer membrane permeability.<sup>103</sup> The enantiomer of PMBN exhibits no activity, highlighting the significance of stereochemical configuration.<sup>104</sup> Despite being less toxic than colistin, PMBN exhibited nephrotoxicity in preclinical studies, hindering its advancement as a therapeutic adjuvant.<sup>105</sup>

### 3.2. Combination therapies and small molecule optimization

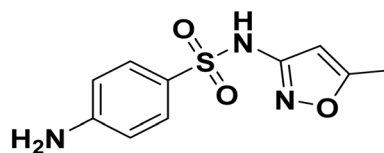
**3.2.1. Combination therapies (synergism).** A strategy to address antibiotic resistance involves the use of combination therapy (CT), which entails administering two or more antibiotics to treat a single infection. CT is applicable to challenging microbial infections that are slow-growing, persistent, extensively drug-resistant, or of unknown etiology, thereby expanding the

antibiotic spectrum.<sup>106–108</sup> CT can yield three primary outcomes on microbes: additive, synergistic, and antagonistic, resulting in effects that are identical, greater, or lesser than the combined effects of individual antibiotics, respectively.<sup>109</sup> Synergistic combinations are frequently pursued in infections characterized by the common occurrence of resistance development and subsequent treatment failure with monotherapy.<sup>110</sup>

**3.2.1.1. Combinations of trimethoprim-sulfamethoxazole (TMP-SMX).** The combination therapy of trimethoprim 42 and sulfamethoxazole 43 targets bacterial folate production by hindering two critical enzymes: trimethoprim blocks dihydrofolate reductase, while sulfamethoxazole inhibits dihydropteroate synthase. By interfering with these sequential steps, the duo effectively halts folate production, essential for bacterial growth and replication. This synergistic antibiotic combination is widely used to treat several infections such as urinary tract infections, respiratory tract infections, and opportunistic infections in patients with weakened immune systems.<sup>111</sup> It is especially important for managing infections triggered by methicillin-resistant *Staphylococcus aureus* (MRSA) and for treating pneumocystis pneumonia.<sup>112</sup>



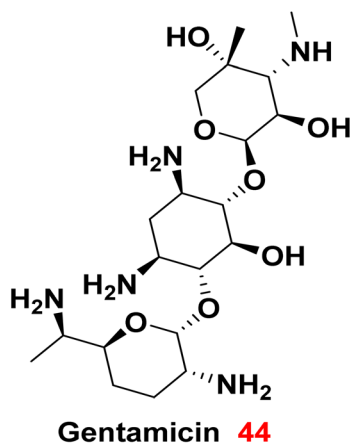
Trimethoprim 42



Sulfamethoxazole 43

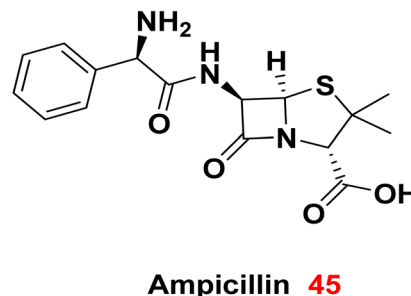


**3.2.1.2. Combinations of aminoglycosides with  $\beta$ -lactam antibiotics.** Aminoglycosides, including gentamicin **44**, can enhance clinical treatment efficacy, expedite bacterial clearance, and bolster antibiotic resistance, particularly when used in association with  $\beta$ -lactam antibiotics like ampicillin **45**.<sup>113,114</sup>  $\beta$ -Lactam antibiotics possess the capability to disrupt the bacterial cell wall, thereby facilitating the entry of aminoglycosides into bacteria and augmenting their bactericidal efficacy. Aminoglycosides combined with  $\beta$ -lactam antibiotics are frequently employed in the treatment of severe hospital-acquired infections caused by multidrug-resistant organisms, including acquired pneumonia, ventilator-associated pneumonia, and sepsis.<sup>115</sup>

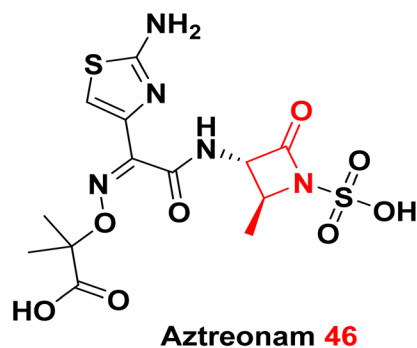


**3.2.2. Small molecule optimization.** The structural modification of established antibiotics is a traditional successful medicinal chemistry strategy to combat resistance by improving target binding, decreasing efflux, or circumventing enzymatic destruction. Ciprofloxacin and its fluoroquinolone analogs are prominent examples that have undergone chemical optimization to regain effectiveness against resistant strains.

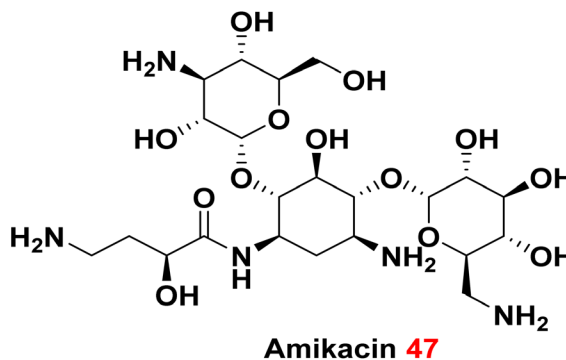
Novel ciprofloxacin derivatives were synthesized in 2021 by reacting ciprofloxacin **48** with various organic reagents. The synthesized compounds were identified through elemental analysis, X-ray analysis, and spectral data. The new derivative, **49**, was exceedingly effective against all the tested organisms. The newly synthesized compounds exhibited excellent efficacy



**3.2.1.3. Combination of monobactams and aminoglycoside.** It was reported that combination of monobactam such as Aztreonam **46** and aminoglycoside such as amikacin **47** gave promising results against metallo- $\beta$ -lactamase-producing multidrug-resistant *P. aeruginosa*.<sup>116</sup>



against ciprofloxacin-resistant *K. pneumoniae* DF72F in comparison to the commercial ciprofloxacin disc (5  $\mu$ g). However, they exhibited moderate efficacy against *E. coli* U65M and *P. aeruginosa* TA74F, which are clinical isolates (Fig. 14).<sup>117</sup>



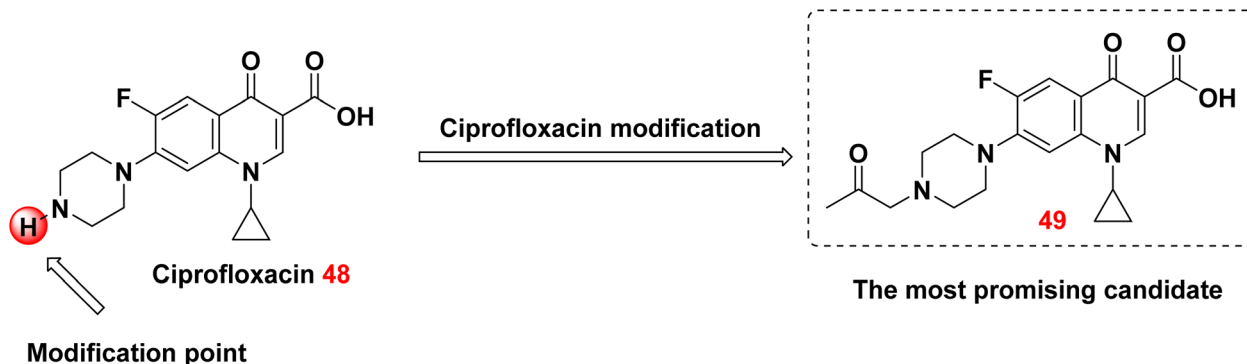
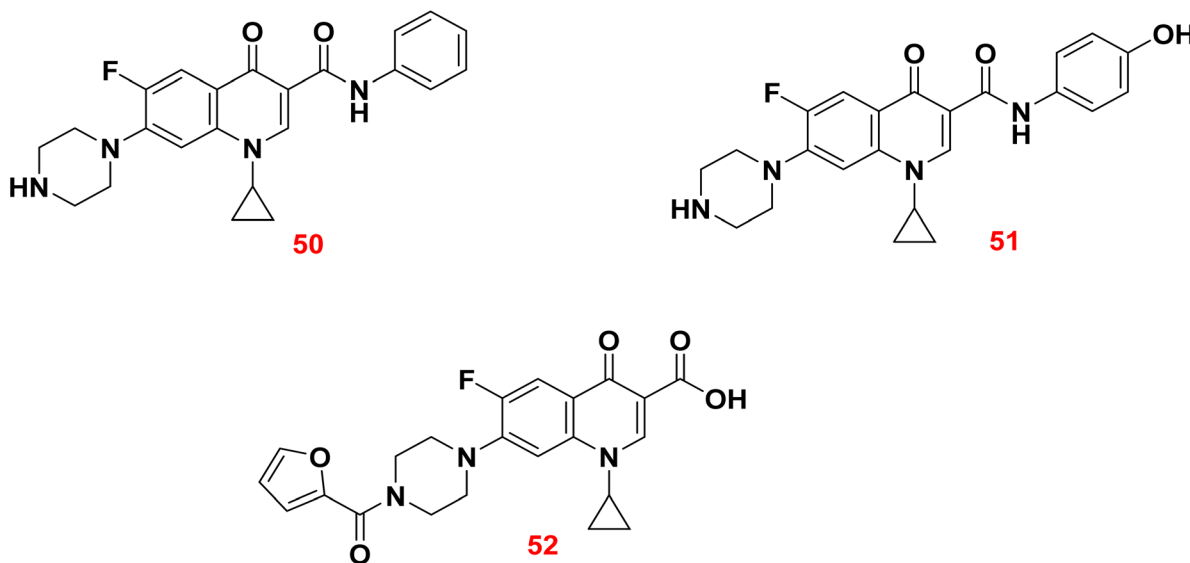


Fig. 14 Structural optimization of ciprofloxacin.

In 2023, six analogs of ciprofloxacin have been synthesized by introducing new functional groups at C-3 and C-7 positions. The antibacterial activity of the synthesized derivatives was assessed against a resistant series of Gram-positive and Gram-negative bacteria using ciprofloxacin as a reference. Among all, the synthesized derivatives **50**, **51**, and **52** showed better activity in comparison to the ciprofloxacin.



### 3.3. Repurposing nonantibiotic drugs as antibacterials

Examining current pharmaceuticals for possible efficacy against drug-resistant bacteria is a viable approach in combating antibiotic resistance. This method entails reassessing existing drugs to see if they exhibit antibacterial capabilities, regardless of their original intent. The repurposing of pharmaceuticals can yield useful therapies for drug-resistant illnesses.<sup>118</sup> An essential benefit of examining current pharmaceuticals is the abundance of safety and effectiveness data accessible. These pharmaceuticals have previously undergone comprehensive testing in

human subjects, which can facilitate their repurposing for antimicrobial uses. Identifying non-traditional antimicrobial compounds inside current pharmaceuticals can augment the antibiotic arsenal and provide novel therapies for drug-resistant microbes.<sup>119</sup>

**3.3.1. Statins.** Because of their possible antimicrobial qualities, statins are frequently recommended to control

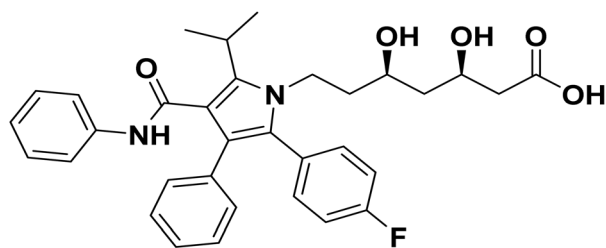
cholesterol levels. Statins have the ability to break down bacterial membranes, which may improve the effectiveness of conventional antibiotics.<sup>120</sup> According to several findings, individuals with bacteremia who had previously had statin medication had a significantly lower death rate than those who had not.<sup>121</sup> Statin therapy was linked in several trials to lower mortality rates in individuals with pneumonia.<sup>122</sup>

It has been established that atorvastatin **53** and simvastatin **54** were more effective against MRSA, vancomycin-resistant enterococcus (VRE), and *Staphylococcus epidermidis*, when

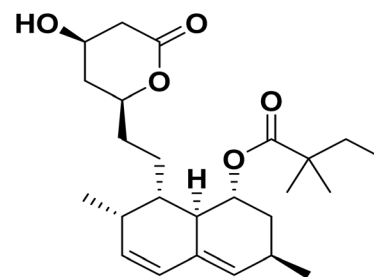


compared to rosuvastatin 55. Conversely, *Enterobacter cloacae* and *E. coli* were more susceptible to Atorvastatin 53 than to simvastatin 54 and rosuvastatin 55.<sup>120</sup>

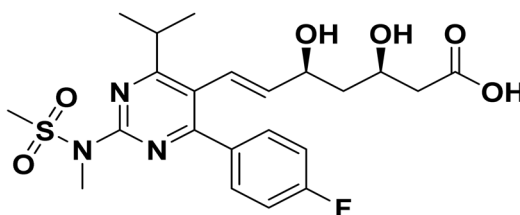
because to the much reduced permeability of its outer membrane compared the other two bacterial species mentioned earlier.<sup>127</sup>



**Atorvastatin 53**

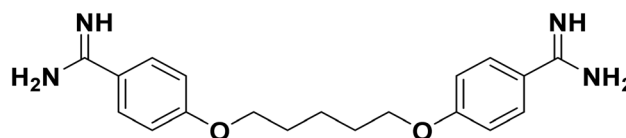


**Simvastatin 54**

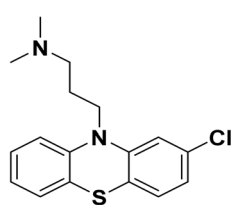


**Rosuvastatin 55**

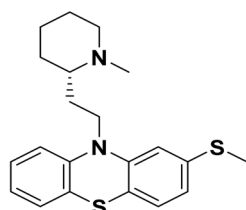
**3.3.2. Antidepressants.** The antibacterial efficacy of antidepressants against multidrug-resistant bacteria has been extensively studied, with many trials showing promising results, especially when combined with standard antibiotics.<sup>123</sup> It was reported that chlorpromazine (CPZ) 56 and thioridazine (TZ) 57 have the capacity to enhance antibiotic susceptibility by blocking bacterial efflux pumps.<sup>124</sup> The susceptibility of MRSA to Oxacillin was proven to be altered by CPZ or TZ, likely due to efflux-related processes.<sup>125</sup>



**Pentamidine 58**



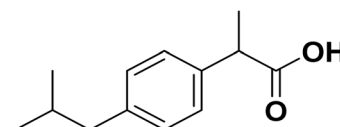
**Chlorpromazine 56**



**Thioridazine 57**

**3.3.3. Pentamidine.** Pentamidine 58, an antiprotozoal drug, exhibited the most significant increase in the outer membrane permeability through disruption of the cation bridge holding the LPS molecules in *E. coli* and *A. baumannii*.<sup>126</sup> It rendered both bacteria susceptible to potent antibiotics such as Novobiocin and Rifampicin. Nonetheless, pentamidine exhibited inactivity against *P. aeruginosa*, may be

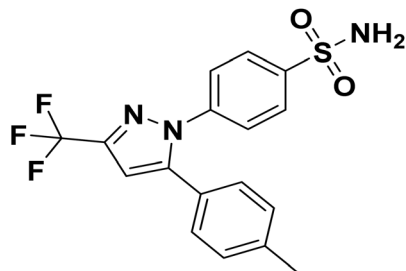
**3.3.4. Ibuprofen.** Recent research indicates that ibuprofen 59 exhibits antitubercular properties, effectively inhibiting the growth of *Mycobacterium tuberculosis* in replicating, non-replicating, and drug-resistant clinical strains. In murine infection models, ibuprofen has demonstrated significant efficacy in reducing bacterial loads in lung tissue, underscoring its value as a complementary therapy for tuberculosis.<sup>128,129</sup>



**ibuprofen 59**

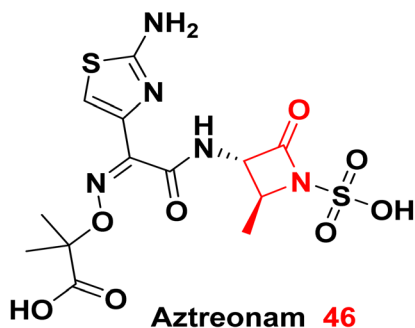
**3.3.5. Celecoxib.** Studies demonstrated that celecoxib 60 enhances bacterial sensitivity to multiple antibiotics by inhibiting multidrug efflux transporters in *S. aureus*. Moreover, Celecoxib exhibited potent efficacy in an animal model of *Caenorhabditis elegans* infected with MRSA.<sup>130</sup>



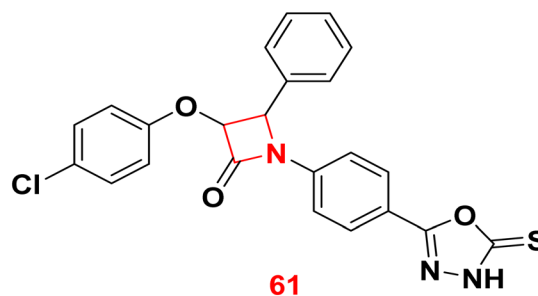


Celecoxib 60

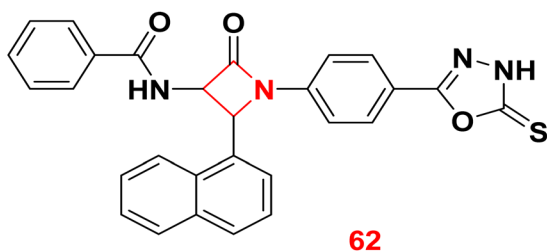
**3.3.6. Limitations of repurposing of nonantibiotic drugs as antibacterials.** Several obstacles and specific issues must be addressed during the repurposing of non-antibiotic medications. While repurposed pharmaceuticals had well-defined toxicological and pharmacological profiles for their initial medical indications, alterations in the administration route or the necessity for combination therapy may require a novel formulation post-



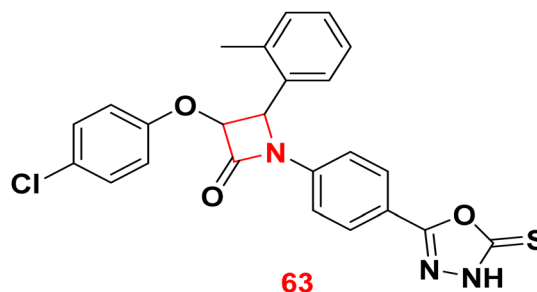
Aztreonam 46



61



62



63

repurposing. In addition, difficulties related to intellectual property rights and potential adverse effects of the major medicinal activities, particularly for anticancer and antipsychotic medications, must be considered. Drug-drug interactions must be considered during repurposing, particularly when therapy involves combination regimens. Additionally, further clinical trials are frequently needed to assess the practical use of novel therapies aimed against bacterial infections.

### 3.4. Improving the profile of existing antibiotic classes

Monocyclic  $\beta$ -lactams, including aztreonam, exhibit greater stability against hydrolysis by  $\beta$ -lactamases in comparison to other  $\beta$ -lactams.<sup>116,131</sup> The monobactams are classified as a subclass of monocyclic beta-lactam moieties.<sup>132</sup> Consequently, concentrating on these monobactam compounds presents a significant opportunity to discover new and innovative  $\beta$ -lactamase inhibitors.

In 2021, a series of monobactam compounds were synthesized and assessed for their  $\beta$ -lactamase inhibitory activities. The MIC was calculated for the monobactam derivatives against four strains of  $\beta$ -lactamase Gram-positive and Gram-negative bacteria. The results obtained were compared with clavulanic acid as a co-inhibitor alongside amoxicillin against the same four strains of bacteria. The biological findings indicated that compounds 61, 62, and 63 exhibited a  $\beta$ -lactamase inhibitory effect against *E. coli* species similar to that of clavulanic acid.<sup>133</sup>

## 4. Discussion and future perspectives

The fight against antibiotic resistance is multidimensional, and while different small-molecule-based treatments have showed promise, each has unique benefits and drawbacks.

### 4.1. Comparison of strategies

- Adjuvants (e.g.,  $\beta$ -lactamase and efflux pump inhibitors) offer a rapid path to clinical utility by enhancing existing antibiotics.



However, their effectiveness is often limited by target specificity and pharmacokinetic mismatches with partner drugs.

- Structural modification of known antibiotics can restore activity against resistant strains while maintaining established safety profiles. However, such modifications may be insufficient to overcome complex resistance mechanisms like efflux pumps or porin loss.

- Drug repurposing provides a cost-effective route by leveraging known drugs, but many repurposed agents exhibit limited antibacterial potency at clinically relevant concentrations.

- Combination therapies can offer synergistic effects, yet they also pose challenges related to dosing, drug–drug interactions, and multi-component resistance.

#### 4.2. Clinical translation bottlenecks

Despite encouraging advances in the design of small-molecule strategies, the successful translation of these candidates into clinical use remains limited. Several key obstacles hinder this process:

- Toxicity of novel adjuvants or dual-target inhibitors (e.g.,  $\beta$ -lactamase and PBP2 inhibitors).

- Poor outer membrane permeability, especially in Gram-negative species.

- Rapid emergence of secondary resistance under selective pressure.

- Regulatory complexity in approving combination or repurposed agents without new clinical trials.

#### 4.3. Emerging and underexplored directions

Several areas hold untapped potential for innovative resistance-fighting solutions:

- Hybrid molecules that chemically link an antibiotic and adjuvant into a single scaffold.

- AI-assisted antibiotic design, accelerating structure-based scaffold development and optimization.

- Membrane-targeting agents and synthetic antimicrobial peptides with non-traditional mechanisms.

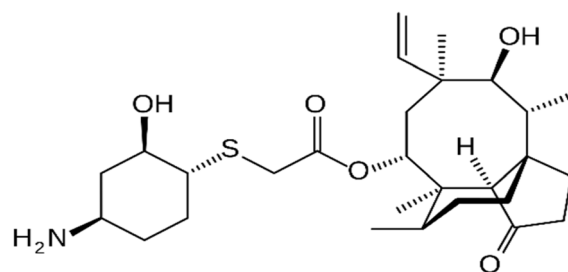
- Non-classical adjuvants, such as quorum sensing inhibitors and antivirulence agents.

In summary, although current methodologies have shown encouraging results, a more profound combination of medicinal chemistry, microbiology, and translational research is essential to transform these techniques into sustainable therapeutic outcomes. The future of antibiotic development depends on adopting complexity through precision-targeted, multifunctional drugs.

## 5. Novel antibiotics targeting drug-resistant bacteria in the recent five years

The development of new antibiotics targeting drug-resistant bacteria represents a vital initiative in addressing the growing issue of antimicrobial resistance. Researchers are diligently investigating novel therapeutic approaches to combat these resilient pathogens.

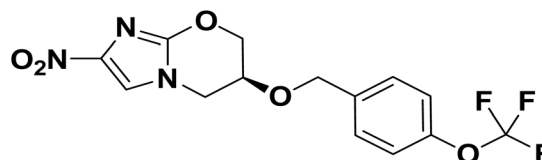
### 5.1. Lefamulin



#### Lefamulin 64

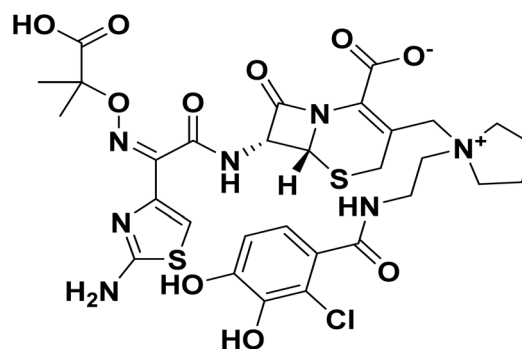
Lefamulin **64** is a pleuromutilin that was approved by the FDA in 2019.<sup>134</sup> Lefamulin is indicated for the treatment of community-acquired bacterial pneumonia, encompassing infections due to drug-resistant *Streptococcus pneumoniae*. This agent inhibits bacterial protein synthesis by binding to the peptidyl transferase center of the 50S bacterial ribosome, thereby preventing the binding of transfer RNA for peptide transfer. It serves as an alternative treatment in cases where resistance to older antibiotics has developed.<sup>135,136</sup>

### 5.2. Pretomanid



#### Pretomanid 65

In 2019, the FDA approved use of Pretomanid **65** with Bedaquiline and Linezolid as a treatment regimen for pulmonary MDR TB. Pretomanid **65** is also used for treatment of extensive drug-resistant tuberculosis (XDR-TB) in adult patients.<sup>137</sup> The mechanism of action involves the inhibition of mycolic acid biosynthesis (similar to isoniazid) to disrupt cell wall formation.<sup>138</sup>



#### Cefiderocol 66



### 5.3. Cefiderocol

Cefiderocol **66** is a broad-spectrum cephalosporin antibiotic. It has been approved in 2019 by FDA and has marketed in several countries. It is highly effective against many drug-resistant Gram-negative bacteria, including carbapenem-resistant Enterobacteriaceae.<sup>139</sup> Cefiderocol works by disrupting bacterial cell walls and facilitating iron uptake, an innovative approach to addressing antibiotic resistance.<sup>140</sup>

## 6. Conclusion

Antibiotic resistance is becoming a bigger and bigger danger to global health, making therapies that save lives less effective. This study looked at a number of small-molecule-based tactics for fighting bacterial resistance, such as using adjuvants, synergistic drug combinations, repurposed non-antibiotic agents, and changing the structure of current antibiotics. Each of these methods has its own benefits, but they all have big problems when it comes to practical translation, such toxicity, low permeability, or the chance of resistance evolving quickly. We have spoken about structure activity relationships (SAR), mechanistic insights, and biological targets that might help with rational drug design from the point of view of medicinal chemistry. To come up with strong and long-lasting remedies, we need to know more about how resistance works at the molecular level. The use of new technologies like AI-driven compound design, hybrid molecule engineering, and innovative target identification will be highly important in speeding up the search for next-generation antibiotics. Continued interdisciplinary collaboration among chemists, microbiologists, and clinicians is crucial to overcome current bottlenecks and to preserve the efficacy of antimicrobial agents in the face of evolving bacterial threats.

## Data availability

This article is a review of existing literature, and no primary datasets were generated or analyzed during the preparation of this manuscript. All referenced data and materials are available in the cited sources.

## Conflicts of interest

There are no conflicts to declare.

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