

REVIEW

[View Article Online](#)
[View Journal](#)Cite this: DOI: 10.1039/
d3md00036bSmall-molecule inhibitors of bacterial-producing metallo- β -lactamases: insights into their resistance mechanisms and biochemical analyses of their activitiesYusuf Oloruntoyin Ayipo, ^{ab} Chien Fung Chong ^c and Mohd Nizam Mordī^a

Antibiotic resistance (AR) remains one of the major threats to the global healthcare system, which is associated with alarming morbidity and mortality rates. The defence mechanisms of *Enterobacteriaceae* to antibiotics occur through several pathways including the production of metallo- β -lactamases (MBLs). The carbapenemases, notably, New Delhi MBL (NDM), imipenemase (IMP), and Verona integron-encoded MBL (VIM), represent the critical MBLs implicated in AR pathogenesis and are responsible for the worst AR-related clinical conditions, but there are no approved inhibitors to date, which needs to be urgently addressed. Presently, the available antibiotics including the most active β -lactam-types are subjected to deactivation and degradation by the notorious superbug-produced enzymes. Progressively, scientists have devoted their efforts to curbing this global menace, and consequently a systematic overview on this topic can aid the timely development of effective therapeutics. In this review, diagnostic strategies for MBL strains and biochemical analyses of potent small-molecule inhibitors from experimental reports (2020-date) are overviewed. Notably, N1 and N2 from natural sources, S3–S7, S9 and S10 and S13–S16 from synthetic routes displayed the most potent broad-spectrum inhibition with ideal safety profiles. Their mechanisms of action include metal sequestration from and multi-dimensional binding to the MBL active pockets. Presently, some β -lactamase (BL)/MBL inhibitors have reached the clinical trial stage. This synopsis represents a model for future translational studies towards the discovery of effective therapeutics to overcome the challenges of AR.

Received 22nd January 2023,
Accepted 31st March 2023

DOI: 10.1039/d3md00036b

rsc.li/medchem

Introduction to antimicrobial resistance (AR)

Antibiotic resistance (AR) in clinically relevant bacteria is a global crisis, which poses a significant threat to human health and therapeutic challenges.^{1,2} This ancient and widespread phenomenon has contributed to a drastic rise in financial burden, especially in the healthcare sector, on account of the prolonged hospitalization of patients. Also, it has been attributed to increased patient mortality and morbidity, owing to the limited therapeutic options available against resistant microbes at present.^{3–6} Recently, the Centers for Disease Control and Prevention (CDC) announced that in 2019, at least 44 000 AR-related deaths occurred, marking a 2-fold increase in the death count compared to that in 2013



Yusuf Oloruntoyin Ayipo

Yusuf Oloruntoyin Ayipo is currently a PhD student in the field of Medicinal Chemistry and is supervised by Professor Mohd Nizam Mordī at the Centre for Drug Research Universiti Sains Malaysia. His research interests include computer-aided drug design, synthesis and biological studies of bioactive molecules for neurological disorders, cancers and microbial diseases. His academic excellence has attracted reputable awards and

scholarships including a PhD. Scholarship by TETFUND Nigeria (2019), Graduate Assistantship, Universiti Sains Malaysia (2019–2022), Best Poster Presenter, CDR Colloquium, Universiti Sains Malaysia (2019), Sci-Mix Presenter, ACS SciMeeting, Atlanta (Fall 2021) and Sanggar Sanjung Award, Universiti Sains Malaysia, 2022.

^a Centre for Drug Research, Universiti Sains Malaysia, USM, 11800, Pulau Pinang, Malaysia^b Department of Chemistry and Industrial Chemistry, Kwara State University, P. M. B., 1530, Malete, Ilorin, Nigeria. E-mail: yusuf.ayipo@kwasu.edu.ng^c Department of Allied Health Sciences, Universiti Tunku Abdul Rahman, 31900 Kampar, Perak, Malaysia

of 23 000.⁷ Consequently, if efforts are not promptly devoted to addressing this issue, bacterial pathogens may evolve into a much more dangerous form, resulting in approximately 10 million annually deaths by 2050.^{8,9} This estimated number of death exceeds the number of cancer deaths, and the extent of the problem has even been recognized by the World Health Organization (WHO), which projected the urgency of this situation.¹⁰

The 'golden era' of antibiotics spanned from 1940 to 1960; however, it was short-lived due to the evolution of bacteria into multi-drug resistance (MDR) or "superbugs".⁴ Natural resistance develops gradually over time as a consequence of genetic changes; however, antibiotic overuse and poor stewardship in medical prescription can also accelerate this unpleasant process.^{3,11} Under antibiotic effects, susceptible bacterial strains are eliminated or inhibited, but antibiotic-resistant strains can prevail. Worryingly, MDR bacteria can spread quickly through a community by plasmid-mediated transfer of AR genes across bacteria of the same or other species.¹² In most cases, immunocompromised individuals and those on intubation are understood to have a high chance of exposure to infection.¹³ However, despite the timely development of new antibiotics against emerging strains, bacteria swiftly develop sophisticated and unpredictable resistance mechanisms to evade the antibacterial actions of new drugs.¹⁰

To date, numerous studies have revealed the common resistance routes shared among bacteria species. The defence mechanisms of *Enterobacteriaceae* (superbugs) to antibiotics understandably occur through several pathways including the overexpression of efflux pumps, mutation of target genes, production of biofilms and most relevantly metallo- β -lactamases (MBLs).¹⁴ Indeed, the beta (β)-lactam class of antibiotics is the main medicine used to counteract various bacterial infections and diseases in critical degrees. However,

drug-resistant bacteria, as a defence mechanism, produce the notorious MBLs, which deactivate antibiotics. The affinity of the BLs for the β -lactam rings contained in antibiotics depends on the ease of formation of stable complexes facilitated by the enzyme cofactors.¹⁵ The carbapenemases, notably, the New Delhi MBL (NDM), imipenemase (IMP), Verona integron-encoded MBL (VIM), *Klebsiella pneumoniae* carbapenemase (KPC) and OXA-48, a D class carbapenemase, represent the critically implicated bacterial enzymes in AR pathogenesis.^{16–18} They are responsible for the worst AR-related clinical conditions, but to date, there are no approved inhibitors, making this an urgent issue to be addressed. The available antibiotics, specifically the most active β -lactam-types, are subjected to deactivation by the notorious enzymes through the opening of their β -lactam rings and deacetylation of their corresponding complexes with the enzymes.^{14,15,19,20} Progressively, scientists have devoted their efforts to curbing this global menace, and therefore a regular overview on this topic can aid in the required timely development of effective therapeutics. Among the recent reviews, Arshada and Hanif surveyed the chelating effects of some natural products, *i.e.*, hydroxypyridone and hydroxythiopyridone derivatives, against several metalloenzymes implicated in cancer, diabetes and microbial diseases, including histone deacetylase (HDAC), tyrosinase and MBL.²¹ However, several other small-molecule scaffolds with more potent activities against specifically MBLs were overlooked. Canton and co-workers also reviewed the resistance mechanisms of *Pseudomonas aeruginosa* and promoted the potency of siderophore cephalosporin cefiderocol as a treatment option in clinical conditions based on previous preclinical investigations.²² Similarly, the genetic background and resistance mechanisms of *Acinetobacter baumannii* to various antibiotic agents through MDR genes were overviewed.³ However, the studies were mainly organism-based, and other carbapenem-resistant bacterial



Chong Chien Fung

Chong Chien Fung is an emerging scholar with a Bachelor's Degree in Biomedical Science from Universiti Tunku Abdul Rahman, Malaysia. His research interests include biochemistry and clinical immunology, with particular emphasis on small G-protein and cancer signalling. Currently he is a Master's candidate and actively engaged in research on the discovery of novel biomarkers.

He is not only passionate about learning and staying up-to-date with the latest developments, but also exploring various opportunities to contribute to this field. He is committed to pursuing a career in cancer research and seeks to collaborate with experienced researchers.



Mohd Nizam Mordi

Mohd Nizam Mordi received his PhD in Medicinal Chemistry from the University of Manchester in 2001. In the same year, he began his journey as a Junior Lecturer Cum Researcher at the Centre for Drug Research, Universiti Sains Malaysia. He has been a Professor in Medicinal Chemistry since 2016. His research endeavours are focused on drug discovery, design, and synthesis. With a computer-aided drug design

technique, his group successfully enhances the discovery of new useful bioactive compounds, in particular heterocyclic compounds that are active for analgesia, antidepressant, anticancer, and addiction treatment.

strains were barely discussed, and relevantly MBL inhibition was underexplored. In our previous work, Ayipo *et al.* described the coordination chemistry of metalloenzymes with substrates and reported some potent metal-chelating inhibitors for the catalytic deactivation of MBLs.¹⁴ However, due to the incessant resistance developed the superbug-produced enzymes and the dynamic evolution of therapeutic strategies, a regular survey of the state-of-the-art in this important subject remains crucial for overcoming the associated global threats. Moreover, hundreds of progressive studies have been reported to date.

In this review, the recent scientific literature on potent small-molecule inhibitors on MBLs as promising therapeutic interventions for AR are comprehensively surveyed. The bacterial resistance mechanisms towards the β -lactam antibiotics and various types of BLs are outlined. In addition, the appropriate diagnostic interventions for MBL are enumerated. In this review, we focus on one of the resistance mechanisms and their therapeutic pathways, *i.e.*, MBL production, specifically implicating MBLs. The ongoing research on various MBL inhibitors through rigorous preclinical and clinical studies can facilitate the development of better treatment regimens for overcoming the therapeutic challenges posed by AR.

Mechanisms of resistance development by bacteria

Microbes are understood to produce systemic antibiotics internally and combat the host microorganism to evade the therapeutic action of this self-produced drug, which can easily facilitate the development of resistance. For instance, thienamycin, isolated from *Streptomyces cattleya*, is an example of a naturally occurring potent β -lactam antibiotic in bacteria.²³ In this case, the co-resident bacteria evolved spontaneously to achieve intrinsic resistance toward certain antibiotics or antibiotic families with similar molecular effects. In the case of acquired resistance, bacteria that were formerly susceptible to an antibiotic or antibiotic family gained the ability to adapt to antibacterial action through an evolutionary process. This can be accomplished through either gene mutation to bacterial chromosomal DNA²⁴ or horizontal resistance gene transfer.²⁵ In summary, the development of MDR by the well-known Gram-negative bacteria including *K. pneumonia*, *A. baumannii*, *P. aeruginosa* and *Enterobacter* species and some Gram-positive with which they constitute the ESKAPE pathogens, *i.e.*, *Enterococcus faecium* and *Staphylococcus aureus*, are facilitated by multifaceted mechanisms, constituting treatment challenges.²⁶

Resistance of *Enterobacteriaceae*

Enterobacteriaceae are a vast family of Gram-negative, facultative anaerobe bacteria, mostly of the order *Enterobacterales*. In recent years, many familiar genera have been documented to have a high frequency of β -lactam

resistance in various regions of the world, including the well-known *Klebsiella* spp.,^{27–29} *Escherichia* spp.,^{28,30} and *Salmonella* spp.³¹ This review provides a progressive understanding of how the emerging threat, *Enterobacteriaceae*-resistant strain, develops resistance against the clinically important antibiotics of the β -lactam family. This covers the biochemical route, including restriction of drug uptake, overexpression of drug efflux pumps, modification of drug target, production of biofilm and drug inactivation by enzymatic hydrolysis implicating MBLs.

Restriction of drug uptake (alteration of bacterial membrane permeability)

Gram-negative bacteria vary from Gram-positive bacteria by their outer layer membrane, which is made up of glycerol phospholipid and glycolipid lipopolysaccharide (LPS).³² The LPS acts as the first line of defence for bacteria against harmful chemicals, notably hydrophilic antibiotics. The propensity of hydrophilic β -lactams to pass through the bacterial outer membrane is dependent on the membrane-embedded specific β -barrel protein channel.³³ Thus, this protein, which is also referred to as a porin, can limit β -lactam uptake by *Enterobacteriaceae* by reducing the number of porins or changing the selectivity of the porin channel.^{28,34} For example, most *Enterobacteriaceae* produce two significant outer membrane proteins (Omps), *i.e.*, OmpC and OmpF, which are regulated by the *ompC* and *ompF* genes, respectively. According to the epidemiological study by Liu and colleagues, low expression of the *ompC* and *ompF* genes is associated with a decrease in carbapenem susceptibility against the *Enterobacter cloacae* complex (ECC).³⁵ Although, another study contradicts this claim, with no significant difference found between the minimum inhibitory concentration (MIC) of tazobactam-piperacillin-resistant *E. coli* and *omp*-deleting mutants.³⁶ Specifically, changes in membrane permeability prevent certain β -lactams, but not all antibiotics, from entering bacterial cells and exerting antibacterial action. However, it can be induced by other connected resistance mechanisms.

Overexpression of drug efflux pumps

The efflux pump genes are encoded on bacterial chromosomal DNA, and the encoded pump tends to remove harmful chemicals. This is one of the mechanisms by which Gram-negative bacteria exhibit AR. When organisms are exposed to β -lactam-produced antibiotics stress, they up-regulate the gene encoding the efflux pump.³⁷ Consequently, overexpression of the pump aids in the removal of antibiotics from the bacterial cells and lessens bactericidal activity. There are five prominent efflux pump families, with the resistance-nodulation-cell division (RND) family abundantly found in the clinically relevant Gram-negative bacteria.³⁴ For instance, some *Enterobacteriaceae* including *E. coli* and *K. pneumonia* express the RND-based tripartite efflux pump, AcrAB-TolC.¹² Suzuki and coworkers reported that the

overproduction of the efflux AcrAB in *E. coli* clinical isolates, combined with the simultaneous overproduction of BLs, results in tazobactam–piperacillin resistance.³⁶ According to Lomovskaya *et al.*, after the deletion of AcrAB-TolC in *K. pneumonia*, the potency of the majority of β -lactam antibiotics increased by 2- to 16-fold,³⁸ indicating the defensive function of the pump against the drugs. These studies consistently documented how the efflux pump influences the efficacy of β -lactam antibiotics, as well as understanding the influences of β -lactam susceptibility in *Enterobacteriaceae* based on more than one resistance mechanism.

Modification of drug targets (alteration of the bacterial protein)

Antibiotics target different components of the bacterial cell depending on the drug class. In this case, the β -lactam class specifically targets the penicillin-binding proteins (PBPs) located on the inner membrane of the bacteria cell wall. This affects the peptidoglycan cross-linking, which is vital for the stiffness and strength of the bacterial cell walls. The resultant effects include an imbalance in the cell wall metabolism, leading to cell lysis.³ This indicates that a change in PBPs can facilitate bacterial resistance to β -lactam antibiotics in two distinct ways. Firstly, by changing the structure of PBPs to express low-affinity for β -lactam,¹⁵ and secondly by modulating the number of PBPs through the up-regulation of low-affinity PBPs or down-regulation of PBPs with normal β -lactam affinity.³⁴ Although this mechanism is uncommon in Gram-negative rod bacteria, some well-known bacteria under this classification such as *Salmonella* spp. and *Escherichia* spp. express it. In another study, Pan and colleagues demonstrated that PBP2 confers β -lactam resistance in the Gram-negative *P. aeruginosa*.³⁹ It was confirmed that when a missense mutation occurs in the gene encoding PBP2, *pbpA*, specifically in the Asp 351 and Val 598 region, the resistance to zidebactam increases. According to Zalacain and colleagues, MBL inhibitor-meropenem combination therapy is less effective in resistant *E. coli* with PBP3 mutation (4 amino acid insertion).⁴⁰ In both studies, the modification of PBPs was believed to play a significant role in β -lactam resistance, and thus represents new clinical relevance to the AR mechanism.

Production of biofilm

The ability to form biofilms is a virulence factor shared by the majority of pathogenic microbes. Bacteria can exchange genetic materials between cells in the slimy matrix, including the resistance gene, which is related to AR mechanisms.³⁴ Generally, bacteria in the planktonic state are susceptible to antibiotics, but under constant antibiotic stress, biofilm formation can be induced to mitigate the drug influence.⁴¹ In this case, one or more types of microbes form biofilms, and the bacteria communities communicate using the resultant quorum sensing (QS) system.⁴² Gram-negative bacteria have genes such as *luxS* for a product known as

autoinducer-2 (AI-2), which has been linked to biofilm production under extreme conditions.⁴³ According to Qian *et al.*, there is a strong association between biofilm-forming ability in *E. coli* clinical isolates and resistance to cephalosporins such as ceftriaxone, cefoxitin, and cefazolin.⁴⁴ Similarly, Al-Bayati and Samarasinghe investigated that biofilm formation was related to carbapenem resistance.⁴³ The *luxS* gene was also highly expressed by *E. coli* and *K. pneumonia* throughout the biofilm formation process, demonstrating the importance of the QS system in biofilm formation. During the biofilm maturation stage, the upregulation of the carbapenemase gene (*bla_{NDM}*) becomes noticeable. These findings suggest that biofilm-forming ability is an AR mechanism that induces efficacious challenges associated with β -lactam antibiotics and it can promote resistance gene exchange among bacteria species.

Drug inactivation by enzymatic hydrolysis (production of β -lactamases)

Bacteria can inactivate antibiotic medication by either degrading it or transferring a chemical group to it, thereby remodifying its efficacy-inclined structure.³⁴ Since the first discovery of penicillin, AR developed due to the hydrolytic activity of bacterial enzymes towards the β -lactam moiety. This action is carried out by the BLs, which hydrolyzes the β -lactam ring, inactivating them before they reach their targets. The majority of clinically relevant Gram-negative bacteria were discovered to employ this as a common mechanism to exert β -lactam resistance.^{10,15,40} The *bla* genes are universal genes that produce BLs, and there is a wide variety of subtypes that target various β -lactams, which will be discussed in the following section. Sreenivasan and coworkers reported a 16-fold difference in the MIC between the β -lactam-MBL inhibitor and β -lactams alone against Gram-negative bacteria.⁴⁵ Furthermore, Lomovskaya *et al.* noted a 4-fold difference in the MIC of a certain cephalosporin between the *K. pneumonia* NDM-1 strain and the non-resistant strain.³⁸ These studies highlight the connection between BL production by *Enterobacteriaceae* and the promotion of β -lactam resistance. A summary of the some common resistance mechanisms developed by the MDR bacterial pathogens is illustrated in Fig. 1.

Beta-lactamases: evolution, epidemiology and classification

The first BLs, AmpC cephalosporinase (chromosomally encoded), were isolated from *E. coli* in the early 1940s.^{4,46,47} As early BLs, they contributed to the development of resistance toward the first β -lactam antibiotic, penicillin. Two decades later, scientists discovered an *E. coli*-derived penicillinase called TEM-1, which is part of a novel category of transferable narrow-spectrum BL (NSBL). Despite its limited ability to hydrolyze β -lactams, it was encoded on a plasmid, making the *bla* gene highly transmissible and can

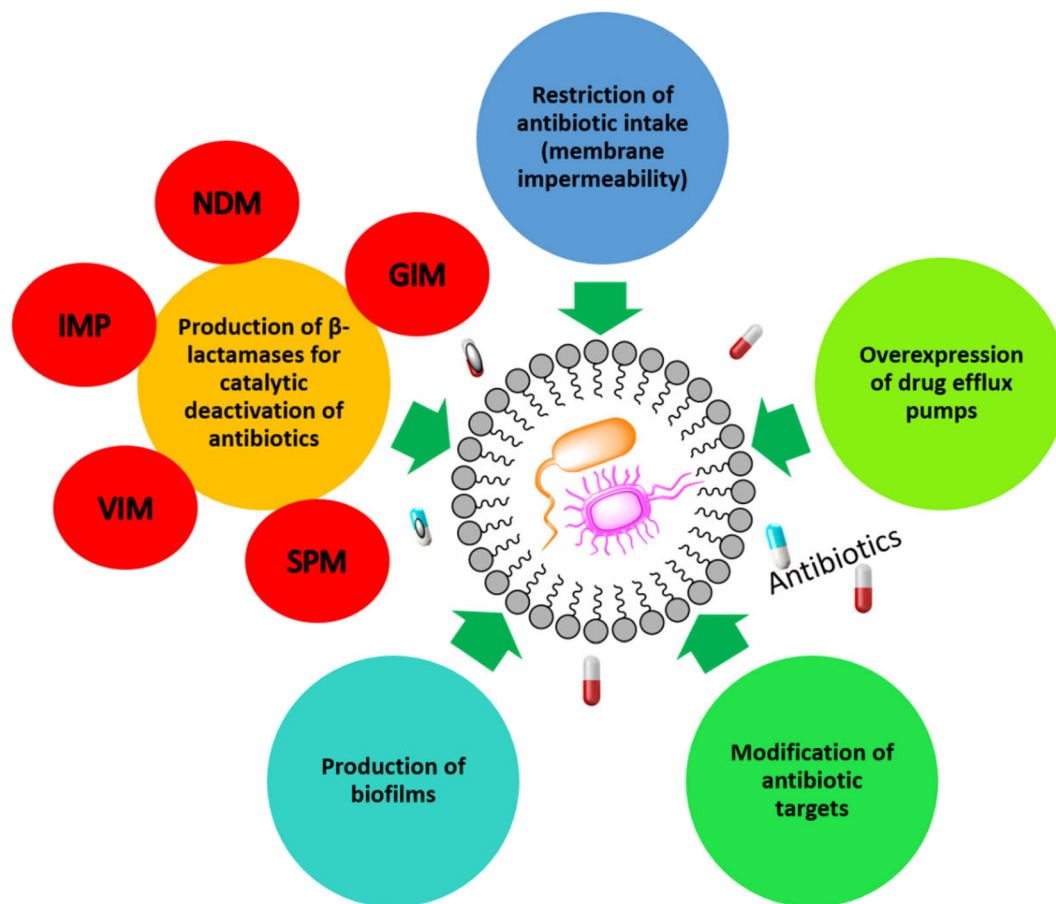


Fig. 1 Illustration of some relevant mechanisms of antibiotic resistance developed by bacteria strains, particularly the production of B-type metallo- β -lactamases.

be freely disseminated among most bacteria.⁴⁷ As an outcome of transferable BLs, a new category of resistance isolates known as extended-spectrum BLs (ESBLs) emerged in the mid-1980s. These ESBLs are more capable to induce β -lactam hydrolysis than NSBLs, comprising the TEM-3 or TEM-52 variant derived from TEM-1.³⁷ In Japan, a new category of plasmid-encoded MBL, imipenemase (IMP), was discovered in 1988 (ref. 12) with stronger influence on AR development than the previous types.

Approximately 890 BLs with varying protein sequences had been identified by 2009.⁴⁸ Accordingly, Bush and Jacoby predicted that BLs will continue to evolve due to major factors such as the spread of mobile drug-resistant genes (plasmid-encoded) to new hosts *via* horizontal gene transfer.⁴⁸ Currently, the number of known BLs exceeds 2700, each with its unique genetic makeup and substrate profile.^{49,50} This statistic depicts the incessant and rapid adaptation of bacteria to confer AR to the relevant β -lactam antibiotics. Coincidentally, the primary mechanism of β -lactam antibiotic resistance in Gram-negative bacteria is the presence of these versatile metal-cofactor enzymes. However, considering the widespread clinical use of β -lactam classes and the rise of resistance in *Enterobacteriaceae*, there is still no successful development of MBL inhibitors.⁵¹

Hence, a thorough understanding and investigation in this area can support the development of better BL inhibitors (BLIs) to counteract these hydrolytic enzymes.

Metallo- β -lactamases

Carbapenems were once thought to be a last-resort treatment due to their ability to avoid the hydrolysis activity of serine-based BLs.¹⁸ However, this was not until they were discovered to be no longer effective against the carbapenem-resistant *Enterobacteriaceae* (CRE), which are capable of producing carbapenemase.⁵² Carbapenemase can be classified as serine-based (Class A and D) and metallo-based Class B.⁵³ The Class B carbapenemase, also widely recognized as MBL, is now relevant because MBL-producing bacteria possess multiple resistance mechanisms. Notably, MBLs can hydrolyze penicillins, cephalosporins, and carbapenems, which limits the therapeutic options for infected individuals. Generally, they are zinc-dependent enzymes that hydrolytically degrade β -lactam antibiotics utilizing the co-factor zinc ions in their active sites.⁵¹ The enzymes in Class B are further classified as B1, B2, B3, B1-like and B3-like based on their amino acid sequence homology and zinc ion content.^{54,55} B1 and B3 contain two Zn ions and can ably target the majority of the

currently available β -lactams, whereas B2, with only one Zn ion, has limited hydrolyzing effect on drugs.⁵⁰ Among these classes, the major representatives of subclass B1 are IMP, NDM, VIM and many more.¹⁶

Imipenemase

The first IMP variant, IMP-1, appeared in 1988. At this time, up to 98 variants had been documented, according to the available data.^{56,57} This plasmid-encoded MBL (*bla*_{IMP}) can spread resistance to other bacteria species *via* class I integron³³ and has previously resulted in an endemic to a specific geographical area.⁴⁷ Primarily, IMP was traced to nations including Japan, Argentina, and Thailand.⁵⁸ However, it is less frequently found in clinical isolates at present. IMP-type MBL is not only found in hospitals but also in the general public.¹⁹ Common Gram-negative bacteria that produce IMP includes *Klebsiella* spp.,⁵⁹ *Enterobacter* spp.,⁶⁰ and *Pseudomonas* spp.⁶¹ Alarming, they have a broad substrate profile, which includes the carbapenems, and the available serine BLIs (clavulanate, avibactam, and vaborbactam) are ineffective against IMP.⁶²

New-Delhi metallo- β -lactamase

The first NDM-type variant, NDM-1, was reported in 2008, which is now regarded as one of the most prominent MBLs.^{63,64} Up to 48 variants have been identified thus far, with some of them being potentially destructive toward carbapenem.^{56,57,65} NDM is primarily known in regions such as India and Pakistan.⁵⁸ Its large substrate profile and widespread occurrence make it a major concern. Except for aztreonam, bacteria carrying NDM can inactivate nearly all β -lactams because the substrate-binding capsule cavity of this enzyme is massive and shallow.^{12,50} Consequently, patients infected with this clinical strain of NDM frequently show a high chance of mortality.²⁵ The plasmid-encoded *bla*_{NDM} gene, which is responsible for NDM enzymes, has been identified in water samples, and it is expected that this gene will spread across bacteria in the natural environment,⁴⁷ making its epidemiology alarming. The *Enterobacteriaceae* that are found to have the NDM include *Klebsiella* spp., *Escherichia* spp., and many more.^{25,50} In recognizing their global threats, they are categorised under the WHO critical pathogens list (Priority 1) as bacteria that mostly produce NDM BLs.⁶⁶

Verona integron-encoded metallo- β -lactamase (VIM) type

The plasmid-encoded MBL, VIM type, was first identified in 1996,⁶⁶ and both VIM-1/2 variants are now regarded as clinically distressing MBLs.^{67,68} An increasing number of VIM variants emerged in *Enterobacteriaceae*, which include *Klebsiella* spp.^{18,50,68} Presently, 83 clinical VIM variants have been discovered worldwide.⁵⁷ This currently represents one

of the prominent types of MBL in the European region,^{66,69} as well as in China, Russia, and Canada.⁵⁸ This *bla*_{VIM} gene is also located on the plasmids or other mobile genetic structures, making it easy to disseminate.^{33,53} Furthermore, the VIM type was observed in fresh meat products in the Egypt region for the first time, raising the alarm of danger to public health services because meats can easily serve as a vehicle for the spread of resistant bacteria.⁵³ Likewise, the substrate hydrolysis profile, which includes penicillin, cephalosporins, and carbapenems but not monobactams, is a point of concern in healthcare settings.¹⁸

Other MBLs: Sao Paulo metallo- β -lactamase type, Germany imipenemase type, and Seoul imipenemase type

Certain Class B1 MBLs are restricted to specific geographical areas and are rarely described, such as the Sao Paulo metallo- β -lactamase (SPM) type, Germany imipenemase (GIM) type, and Seoul imipenemase (SIM) type. SPM-1, GIM-1, and SIM-1 were named after the countries where they were discovered, namely Brazil (1997), Germany (2002), and Seoul (2003), respectively.¹² SIM was isolated from *Acinetobacter baumannii*, whereas SPM and GIM were isolated from *P. aeruginosa*. These carbapenemase encoding genes, *i.e.*, *bla*_{SPM}⁷⁰ and *bla*_{GIM},^{16,70} can also be found in certain *Enterobacteriaceae*, which are not only restricted to *Citrobacter* spp., *Enterobacter* spp., and *Klebsiella* spp. Because they are carbapenemases, they can hydrolyze nearly all β -lactams and are resilient against commercially viable serine BLIs.

Molecular features of B1 subclass of metallo- β -lactamases

Metallo- β -lactamases are ubiquitous, highly promiscuous and mutative enzymes commonly characterized by the ability to hydrolyse almost all generations of β -lactam antibiotics, even in combined forms. They catalyze the hydrolytic cleavage of the β -lactam rings of the antibiotics, rendering them inactive.^{71,72} They naturally possess β -lactamase genes located on a mobile plasmid and can be incorporated into bacterial chromosomes.^{20,71,73} Class B constitutes the most relevant, having varying substrate-binding one/two Zn²⁺ ions, which are essential for catalysis. Their producer pathogens are mostly implicated in bacterial induced deadly infections in clinical settings. In molecular forms, these enzymes have conserved features of $\alpha\beta/\beta\alpha$ MBL sandwich folds as their main scaffold. The B1 subclass possesses a large, open-binding sites in shallow grooves with multiple flexible loops, in addition to the dinuclear Zn ions and water molecules for anchoring the β -lactams for nucleophilic reactions during catalysis.^{55,71} The flexible loops linking the helices along their amino acid chains enhance the stabilization of hydrophobic binding of substrates to the active sites in their

closed forms. Commonly, they have aspartic acid, histidine, asparagine and cysteine as conserved active site residues. The L3 and L10 loops enhance the conformational dynamics of the enzymes for binding different substrates including the bicyclic β -lactams. The loops also bridge some charged amino acid residues that are critical for electrostatic or hydrogen bonding interactions with substrates. The available phenyl alanine and lysine contribute to the hydrophobic interactions of the active pockets with substrates, making them essential for catalysis.^{54,55,74} These molecular features are apparent in the crystal structures of IMP-1 (PDB ID 1JJT),⁷⁵ NDM-1 (PDB ID 5YPL),⁷⁶ VIM-1 (PDB ID 5N5H)⁷⁷ and SPM-1, a hybrid of B1/B2 MBL subclass (PDB ID 5NDB)⁷⁴ (Fig. 2). Due to their multidrug-resistant features, they are not easily deactivated by mechanism-based inhibitors that can form irreversible covalent adducts such as clavulanic acid and tazobactam derivatives.⁷¹ However, they are susceptible to Zn-chelators such as carboxylic acid derivatives, *e.g.*, succinic, salvanolic, picolinic, boronic, phosphonic and rosmarinic acids, and other metal ion sequestrators including hesperidin, aspergillomarasmine and fisetin.^{14,54}

Diagnosis of metallo- β -lactamases

A speedy treatment regimen is normally accompanied by rapid identification of causative agents. In this instance, competent MBL detection facilitates early antimicrobial drug optimization as well as improved infection management strategies. Bianco and collaborators described a new approach for distinguishing MBLs from serine BLs utilizing a direct EDTA-modified- β -lactam inactivation method (deBLIM).⁷⁸ After incubating the three disks (meropenem, MEM; MEM + EDTA; and cefotaxime, CTX) in bacterial suspensions from *Enterobacterales*-positive blood culture, they were placed on a pre-incubated Mueller-Hinton (MH) agar plate (seeded with *E. coli* indicator strain for 2 h). Subsequently, the inhibition zone diameter (IZD) was compared to the negative control. If the isolate contains serine BLs, bacteria grow on the plate, indicating no significant EDTA-dependent inhibition, with IZD_{MEM+EDTA} not exceeding 4 mm compared to the negative control. If the isolate contains MBLs, bacteria cannot grow in the MEM + EDTA disk surroundings, indicating MBL inhibition, with IZD_{MEM+EDTA} exceeding 4 mm compared to the negative control. This method was previously recognized to be cost-effective, and the procedure is simple to implement. A 100% sensitivity rate for aerobic blood culture was obtained regardless of the MBL type; however, its poor performance in detecting MBLs (VIM and NDM) under anaerobic conditions with a range of 56.1% to 80% should be noted.⁷⁸

Recently, the Carba M test for detecting and distinguishing Class A and D carbapenemases (serine BLs) from Class B (MBLs) in Gram-negative clinical isolates was developed.⁷⁹ This biochemical test is based on the colour variation in three tubes containing different reagents, *i.e.*, A (ZnSO₄ + phenol red), B (ZnSO₄ + phenol red + imipenem),

and C (phenol red + imipenem + EDTA), after being inoculated with a direct bacterial colony. The presence of phenol red indicator can identify the presence of bacteria capable of hydrolyzing the β -lactam ring, causing the pH to drop and the solution to change from red to yellow. MBLs that can be hindered by EDTA show no colour change in tube C compared to serine BLs. Serine BLs unaffected by EDTA can hydrolyze imipenem, causing the solution in tube C red to become yellow. The merits of this test include economical reagents that can be found in most lab settings, as well as quick data interpretation for rapid diagnosis. Surprisingly, its 100% sensitivity and specificity demonstrate that the NDM and VIM types can be identified all the time. Even when a single strain produces two MBLs simultaneously, a 100% sensitivity detection rate is still achievable. In comparison to deBLIM, which has 100% sensitivity to oxacillinase (OXA)-48-type serine BLs, Carba M has markedly lower sensitivity (48%) to OXA-48.⁷⁹

Li *et al.* proposed the application of liquid chromatography-coupled tandem mass spectrometry (LC-MS/MS) for the effective identification of various carbapenemases in *Enterobacterales*.⁸⁰ This method employs the concept in which 3-aminophenyl boronic acid (APB) and EDTA inhibit Class A carbapenemases and MBLs, respectively. The principle was primarily based on the measurement of the ratio in the peak area of hydrolyzed and intact meropenem (with or without the presence of an inhibitor). Bacteria colonies were placed in MH broth with meropenem, and then transferred to three tubes containing different components, *i.e.*, A (sterile saline), B (APB), and C (EDTA). Treatment promoted varying degrees of BL inhibition, and then meropenem was monitored by LC-MS/MS analysis. The retention time difference between the intact meropenem (2.03 min) and hydrolyzed meropenem (1.83 min) is minor, but it exists. Given that MBLs were expected to be inhibited in tube C, MBL-producers would have a substantially lower hydrolyzed meropenem peak area than that in tubes A and B. It discriminated between MBLs and Class A carbapenemases based on the inhibitory effect of APB in tube B against the latter. Consequently, compared to tubes A and C, Class A carbapenemases-producer had a relatively smaller peak area for tube B. Interestingly, this approach achieved 100% specificity in detecting Class A carbapenemases and MBL-producing bacteria, with a sensitivity of up to 97.64%.⁸⁰ Despite its speedy and reliable detection, the LC-MS/MS instrument is not widely available in every laboratory setting, limiting its application. However, the MDR pathogens, especially the ESKAPE family are mostly detected in clinical isolates using some of the aforementioned techniques. However, a recent study also reported their prevalence in environmental systems including food, soil, surface water and waste water, making them epidemiological threats.²⁶ Therefore, in addition to clinical diagnosis, regular screening of environments for these deadly microorganisms remains crucial for designing effective therapeutic strategies amenable to curtailing bacterial epidemiology. For the MBLs

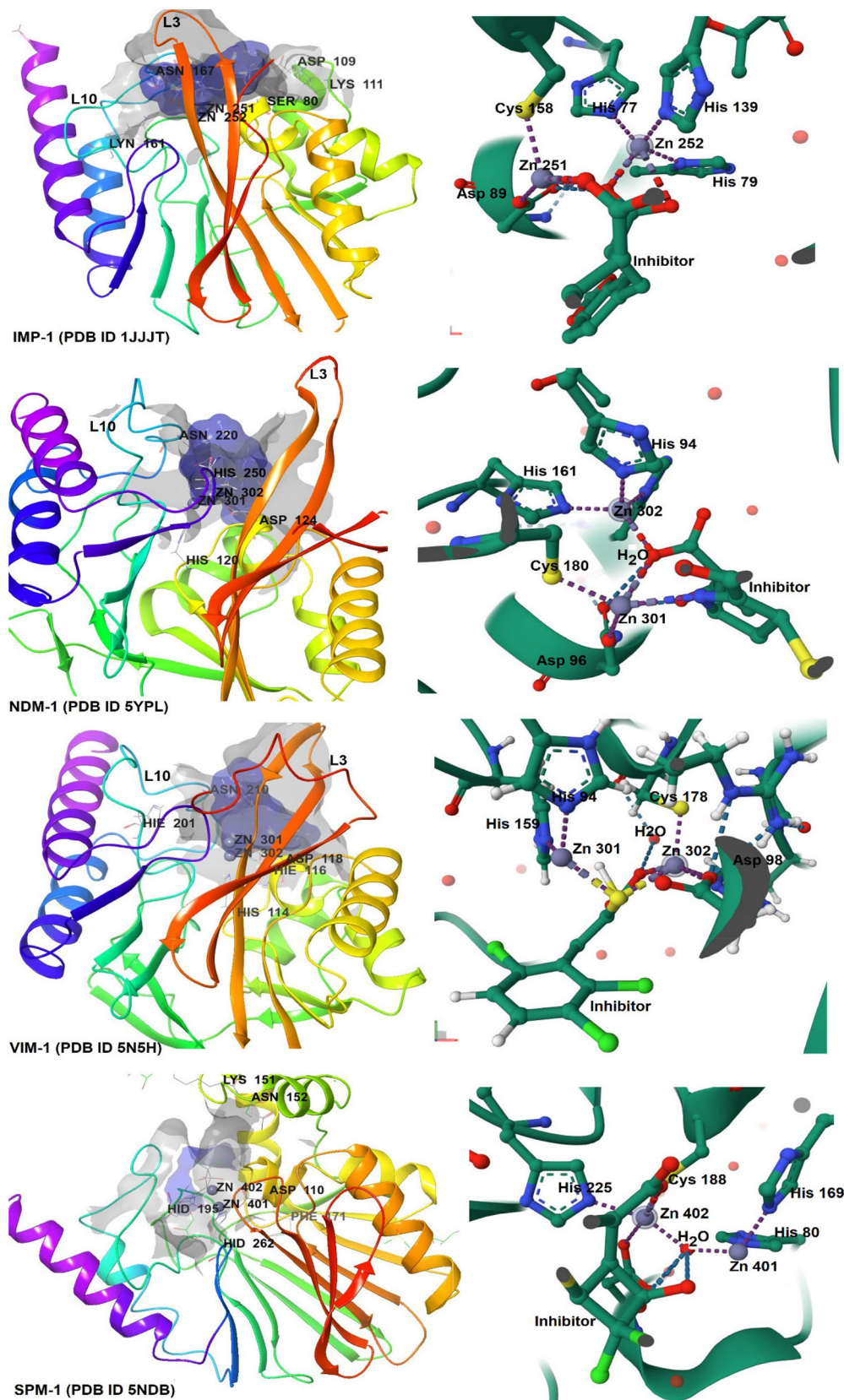


Fig. 2 Active binding pockets of some B1 subclasses of metallo- β -lactamases, IMP-1 (PDB ID 1JJT), NDM-1 (PDB ID 5YPL), VIM-1 (PDB ID 5N5H) and SPM-1 (PDB ID 5NDB), illustrating common features. (Left panel) The surfaces of the active pockets are indicated by blue-grey mesh surrounded by flexible loops linking the helices along their charged amino acid residue chains, which enhance the stabilization of binding of substrates. (Right panel) Coordination of inhibitors with the Zn ions and amino acid residues essentially for mitigating catalysis (images produced using Maestro 12.2, Schrodinger, LCC, New York, NY, 2019 and RCSB ligand interaction diagrams).

produced by *Enterobacterales*, several agents including small-molecules, metal complexes, nanoparticles and peptides have been experimented as potent inhibitors, some of which have deservedly progressed to clinical trials.

Small-molecule inhibitors of MBLs: activity and mechanisms of action

The development of an effective MBL inhibitor is challenging for several reasons including shallow groove, structural diversity and off-target toxicity.⁶⁶ The limitations of therapeutic options for MBL-mediated resistance have caused it to become more prevalent daily and threaten the efficacy of antibiotics including the last-resort β -lactams. Thus, scientists have developed various strategies to overcome these challenges.

Metal ions (Zn) removal or replacement with other metals induce the inactivation of the enzymes, giving rise to the hope of overcoming AR.^{14,66} Strong metal-chelating agents, such as ethylenediaminetetraacetic acid (EDTA), have been shown to inhibit MBLs by removing the metal ions.^{81,82} Relevant studies have also been reported as progressive efforts to develop inhibitory agents using nanoparticles,^{83–85} macrocyclic peptides,⁸⁶ and many others. In this study, an overview of the potent small-molecule inhibitors of various bacterial MBLs reported from 2020 to the present, together with their biochemical analyses and mechanism of action are comprehensively presented.

Inhibitors of imipenemase (IMP)

A study of the synthesized tetradentate Schiff base ligands 2,2'-((1*E*,1'*E*)-(pyridine-2,3-diylbis(azaneylylidene))bis(methaneylylidene))bis(4-bromophenol) (**S1**) and 2,2'-((1*E*,1'*E*)-(pyridine-2,3-diylbis(azaneylylidene))bis(methaneylylidene))diphenol (**S2**) with modest antibacterial properties against bacteria indicated that they have a stable interaction and strong binding affinity for IMP-1. The potent IMP-1 interactions had respective docking scores of -9.8702 and -10.8553 kcal mol⁻¹, as well as the inhibitory concentration at 50% (IC₅₀) values of 83.7 and 93.73 μ M, respectively against the IMP-1 of *A. baumannii*. The **S1**-IMP-1 complex interaction mainly involved Zn1, Zn2, and Asp81, whereas the **S2**-IMP-1 complex primarily involved Zn1, Zn2, and Lys161. In combination with imipenem, the molecules (**S1**-imipenem and **S2**-imipenem) enhanced the efficacy of the antibiotic against MBL-producing *A. baumannii*, as indicated by IZD values of 25 mm and 23 mm, respectively, compared to the EDTA-imipenem combination, which has an IZD of 19 mm. These results indicate that both **S1** and **S2** exhibited 20–30% greater antibiotic effectiveness, targeting IMP-1.³² The docking analysis suggests that the mechanism of action is the deactivation of IMP through inhibition and chelation effects on Zn at the active site induced by the ligands. However, a more rigorous analysis such as *in vivo* analysis can provide more conclusive evidence in this regard.

Ooi and co-workers synthesized a potent 3-(6-aminopyridin-3-yl)-1-sulfamoyl-1*H*-pyrrole-2-carboxylic acid (**S3**) with the ability to target MBLs in carbapenem-resistant *Enterobacterales*. **S3** exhibited an IC₅₀ value of 0.43 μ M against the IMP-1 enzyme. However, this compound showed no significant antibacterial activity independently (MIC > 128 mg L⁻¹). Alternatively, **S3**-meropenem combination therapy reduced the MIC value of an *E. coli* clinical isolate expressing IMP-type from 4 to 0.03 mg L⁻¹ (128-fold reduction), demonstrating an interesting synergistic effect. Its combination with imipenem also resulted in a moderate synergistic effect, given that the MIC decreased from 4 to 0.25 mg L⁻¹ (8-fold reduction). Its inhibition mechanism remains understudied; however, based on the human matrix metalloproteinase inhibition assay, **S3** (at 100 μ M) did not cause off-target toxicity, as supported by the insignificant metalloproteinase inhibition. Also, it had the slightest cytotoxic effect against the HepG2 cell line, where the cytotoxic concentration 50% (CC₅₀) value exceeded the tested concentration (256 mg L⁻¹). In addition to acting on the IMP type, **S3** was also discovered to potently and moderately affect the NDM- and VIM-type, demonstrating its broad spectrum potential activity.⁸⁷

A 2-mercaptomethyl-thiazolidine derivative, (2*S*,4*S*)-2-(ethoxycarbonyl)-2-(mercaptomethyl)-5,5-dimethyl thiazolidine-4-carboxylic acid (**S4**), showed broad inhibitory activities across clinically relevant MBLs (IMP-1, NDM-1, and VIM-2) due to its sulphide- π stacking with conserved MBL active site residues. With an inhibition constant (K_i) value of 2.0 μ M *versus* IMP-1, **S4** exhibited significant inhibitory potential. Its progress curve revealed the mechanism of competitive inhibition, and its crystal structure proved that its thiol (*S*) moiety coordinates with Zn (a metal chelating mechanism). Phenyl alanine-87 was the main interacting conserved residue involved in the hydrophobic interaction between the residues of MBLs and the inhibitor, which stabilized and strengthened the binding. The MIC values of imipenem against the IMP-producing pathogens ranging from 1 to 0.5 mg L⁻¹ against *K. pneumonia*, 16 to 8 mg L⁻¹ against *Enterobacter* spp. and 16 to 8 mg L⁻¹ against *Proteus mirabilis*, respectively, decreased by 2-fold in combination with **S4**, indicating a synergistic effect. Interestingly, the viability of the HEK293 and L929 cell lines remained greater than 85% at concentrations >533 μ M, indicating its negligible cytotoxicity.⁸⁸

Di-2-pyridylketone 4-cyclohexyl-4-methyl-3-thiosemicarbazone (**S5**), a dipyridyl-substituted thiosemicarbazone, was reported as a potent inhibitor of IMP-1 with IC₅₀ and inhibitory concentration-90% (IC₉₀) values of 0.28 and 2.5 μ M, respectively. The inhibition mechanism of Zn-inhibitor complex formation (a metal chelating mechanism) and ability of **S5** to act in a competitive, reversible manner on MBLs was verified, including the generation of Lineweaver-Burk plots and Zn content determination. Supporting its broad-spectrum activity, **S5** not only targeted IMP-1 but also NDM-1 and VIM-2. At a concentration of 4 μ g mL⁻¹, **S5**-meropenem displayed an insignificant synergistic effect against *E. coli* carrying IMP-1

(2-fold reduction in MIC value from 32 to 16 $\mu\text{g mL}^{-1}$). However, the MIC value decreased 8-fold upon the use of 8 $\mu\text{g mL}^{-1}$ concentration. Cytotoxicity testing on the Vero-E6 cell line showed IC_{50} values between 100 to 200 μM , indicating ideal potential for safety and low toxicity.⁸⁹

Two H2dpa derivatives containing pentadentate ligands, (R)-6,6'-(((1-carboxyethyl)azanediyl)bis(methylene))dipicolinic acid (**S6**) and (S)-6,6'-(((1-carboxyethyl)azanediyl)bis(methylene))dipicolinic acid (**S7**), potently inhibited IMP-1 with IC_{50} values of 1.56 and 1.54 μM , and K_i values of 1.57 and 1.54 μM , respectively. In combination, **S6**-meropenem and **S7**-meropenem were effective against multiple IMP-4-producing clinical isolates. This was supported by the significant reduction in minimum inhibitory concentration 90% (MIC_{90}) of meropenem from 1 to 0.25 $\mu\text{g mL}^{-1}$ and from 0.5 to 0.125 $\mu\text{g mL}^{-1}$ in the respective combination. In comparison to the single meropenem therapy, the time-dependent treatment with **S6**-meropenem and **S7**-meropenem resulted in a 10 000-fold reduction in the IMP-4-carrying bacteria population in 24 h. The mechanism of action against VIM type was explored using a Lineweaver-Burk plot and a Zn dependence experiment, suggesting a non-competitive inhibitory effect with zinc chelation (a metal chelating mechanism). Astonishingly, the compounds had relatively safe profiles (haemolytic concentration 50%, HC_{50} value of $>1024 \mu\text{g mL}^{-1}$), and even at high concentrations, exhibited less toxic effects as indicated by the no-observed-adverse-effect level (NOAEL) when tested on human gastric epithelial cells (at 32 $\mu\text{g mL}^{-1}$) or a mouse model (at 50 mg kg^{-1}).⁹⁰ The interesting activities and safety profiles demonstrate the potential of compounds **S6** and **S7** as promising inhibitors of IMP-1 for further studies.

A cephalosporin-thiol conjugate, (6R,7R)-3-(((carboxy(phenyl)methyl)thio)methyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic (**S8**), exhibited potent inhibition of IMP-1 and IMP-28, as indicated by their IC_{50} value of 0.47 and 0.46 μM , respectively. **S8** at 32 $\mu\text{g mL}^{-1}$ decreased the MIC value of meropenem against IMP-1 harbouring *Enterobacter cloacae* by 32-fold reduction (from 32 to 1 $\mu\text{g mL}^{-1}$) and IMP-28 harbouring *K. pneumonia* by ≥ 64 -fold reduction (from 4 to 0.063 $\mu\text{g mL}^{-1}$). Notwithstanding, **S8** showed no significant effect against clinical isolates that expressed VIM-2 and NDM-1. The liquid chromatography-mass spectrometry (LC-MS) and proton nuclear magnetic resonance (^1H NMR) analyses suggested that MBL-induced hydrolysis of the molecule resulted in the release of thiol fragments, which can chelate Zn at the enzymatic site. In conjunction, molecular docking suggested that the IMP-type selectivity may be a result of interactions with Trp28 and Leu165 residues.⁹¹ This study is consistent with the report by Rossi *et al.*,⁸⁸ where the thiol group mainly participated in Zn-chelating effects supported by interactions with Phe 87, both promoting the Zn-sequestration approach for MBL deactivation.

The aryl sulfide-containing 1,2,4-triazole-3-thione compounds, 4-[2-(benzylsulfanyl)ethyl]-3-phenyl-4,5-dihydro-1H-1,2,4-triazole-5-thione (**S9**) and 4-[2-(benzylthio)ethyl]-3-(1-methyl-1H-pyrrol-2-yl)-

4,5-dihydro-1,2,4-triazole-5-thione (**S10**), displayed notable inhibitory effects on IMP-1 with the K_i values of 19.6 and 9.04 μM , respectively. The HeLa cell line screening of these small-molecule inhibitors yielded IC_{50} values of 152 and $>250 \mu\text{M}$, respectively. The treatment of red cell samples with inhibitors yielded IC_{50} values in the range of $>500 \mu\text{M}$, indicating that they have a negligible haemolytic effect at high concentrations. However, **S9** may be off-target due to its IC_{50} value of 52 μM against human glyoxalase II. Moreover, the broad spectrum activity comprising NDM-type (NDM-1) and VIM-type (VIM1, VIM-2, and VIM-4) was also predicted.⁶²

Sometimes, the strong Zn-chelation mechanism is not explicitly a necessity for potent inhibitory activities on MBLs by small-molecule ligands or in synergy with other medications.⁵¹ For instance, 3-(4-fluorophenyl)-7-isopropyl-1H-indole-2-carboxylic acid (**S11**) comprised of an indole-2-carboxylate core showed strong inhibitory activity against IMP-1 quantitatively by IC_{50} value = 0.140 μM . Following the isothermal titration calorimetry (ITC) assay, the compound displayed no significant binding to metals including free Zn, Ca, or Mg ions, indicating that the effective MBL inhibition is does not correlated with substantial binding to Zn at the enzyme site. Because of the absence of metal binding characteristics, this compound is advantageously less prone to induce off-target toxicity. In addition, **S11** was also an NDM-type targeting inhibitor,⁵¹ with a propensity for broad-spectrum effects.

A nitroxoline analogue, 2-(benzyl((8-hydroxy-5-nitroquinolin-7-yl)methyl)amino)acetonitrile (**S12**), was demonstrated as a strong MBL inhibitor, targeting IMP-1 with an IC_{50} value of $<3 \mu\text{M}$. The Zn complementation assay revealed that the mechanism of action was Zn chelation from the MBL enzymatic site (a metal chelating mechanism). **S12**, at 32 mg L^{-1} , has lower bactericidal activity, but when combined with imipenem against clinical strains of *P. aeruginosa* expressing IMP-1 and IMP-7, the MIC values were reduced from 32 to 1 mg L^{-1} (32-fold) and from 128 to 16 mg L^{-1} (8-fold), respectively. However, the safety of **S12** should be considered, given that screening on HUVEC cell lines revealed an IC_{50} value of 45 $\mu\text{g mL}^{-1}$ and the possibility of inducing apoptosis in cells.⁷⁰

Legru and collaborators produced (S)-2-(bis((1H-imidazol-4-yl)methyl)amino)-5-(3-phenyl-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)pentanoic acid (**S13**) with modest inhibitory effect on IMP-type enzymes but broad-spectrum action against NDM- and VIM-type enzymes. Only 40% of IMP-1 is inhibited at a concentration of 100 μM . **S13** is discovered to act in a competitive inhibitory manner by partially stripping Zn from the MBL hydrolytic site (a metal chelating mechanism) using X-ray crystallography, ITC assay, and equilibrium dialysis. IMP-1 was the least affected by this mechanism due to its lower Zn accessibility. Surprisingly, **S13** displayed minimal metabolic instability, no potential for cytotoxicity (IC_{50} values against HeLa cell line $>250 \mu\text{M}$), and low likelihood of off-target effect.⁹²

Another 1H-imidazole-2-carboxylic acid derivative, 1-(4-(thiophen-2-yl)phenethyl)-1H-imidazole-2-carboxylic acid

(S14), inhibited MBLs well. The inhibitory action against purified IMP-1 was satisfactory, with IC_{50} values of 64.02 μ M. However, when tested for meropenem synergism against IMP-1 expressing *E. coli* strains, S29 failed to restore meropenem action.⁹³

Two small molecules, 2,5-dimethyl-4-sulfamoylfuran-3-carboxylic acid (S15) and 2,5-diethyl-1-methyl-4-sulfamoylpyrrole-3-carboxylic acid (S15), both possess sulfamoyl heteroarylcarboxylic acid as their base were reported. S15 inhibits IMP-1 competitively with a K_i value of 0.22 μ M, and S16 inhibits IMP-1 competitively with a K_i value of 0.26 μ M. In the checkerboard assay, which measures apparent synergism, the S15–meropenem combination yielded an FIC index value of 0.11 against the IMP-1-producing engineered *E. coli* strain, whereas the S16–meropenem combination yielded an FIC index value of 0.02. Furthermore, in the presence of meropenem with S15 or S16 (at 4 μ g mL⁻¹), meropenem activity was recovered, with meropenem MIC values dropping by 64- to 128-fold against 19 IMP-1-producing *Enterobacteriaceae* clinical isolates. Nevertheless, the degree of recovery of S15 was not particularly effective against *P. aeruginosa*. After receiving meropenem-inhibitor combination therapy, more than 90% of the murine model (infected with a lethal dose of IMP-1 carrying *E. coli* clinical isolate) survived with S15 (at 10 mg kg⁻¹), while 100% of the murine model survived with S16 (at 10 mg kg⁻¹). X-ray crystallography suggested that S15 interacts mechanically with two Zn ions of MBLs (a metal chelating mechanism) and conserved residues (Lys224 and Arg228). S16, which has a similar base to S15, interacts similarly. Notably, S15 had a negligible off-target effect (IC_{50} value against angiotensin-converting enzyme >1000 μ M) and was non-toxic (HeLa cell line viability at 1000 μ M > 50%; LD₅₀ value on mice model *via* intravenous and intraperitoneal = 246 and >1000 mg kg⁻¹, respectively) and non-mutagenic. S16 was also negligibly (HeLa cell line viability at 1000 μ M > 50%).⁹⁴ A summary of the activity data including mechanisms of actions and safety profiles of selected potent small-molecule inhibitors of IMP curated from the recent literature is presented in Table 1.

Inhibitors of New Delhi metallo- β -lactamase

Several small-molecule inhibitors of NDM-1 have been reported from both natural and synthetic sources. Among the natural products, emerione A (N1) from *Aspergillus nidulans*, was predicted to have the strongest NDM-1 inhibitory potential (docking score = -39.5 kcal mol⁻¹) among other screened compounds in a study. It was observed to interact directly with the Zn of the NDM-1 enzymatic site, a signal for metal chelating mechanism and had a stable binding. *In vitro* studies corroborated the potent inhibitory activity on NDM-1 with an IC_{50} value of 12.1 μ M and equilibrium dissociation constant (K_d) of 11.8 μ M. When N1 was combined with meropenem and imipenem (carbapenem antibiotics), the carbapenem susceptibility on NDM-1-producing *E. coli* and *K.*

Table 1 Summary of selected potent small-molecule inhibitors of IMP, their activities, mechanisms of actions and safety profiles

Small molecule	Chemical analogue	Mechanism of action	Inhibitory activity	Target	Safety profile	Ref.
S1	Phenol	Metal chelation	IC_{50} value = 83.7 μ M IZD _{imipenem+S1} value = 25 mm	IMP-1 MBLs-producing <i>A. baumannii</i>	N/A	32
S2	Phenol	Metal chelation	IC_{50} value = 93.73 μ M IZD _{imipenem+S2} value = 23 mm	IMP-1 IMP-type-producing <i>A. baumannii</i>	N/A	32

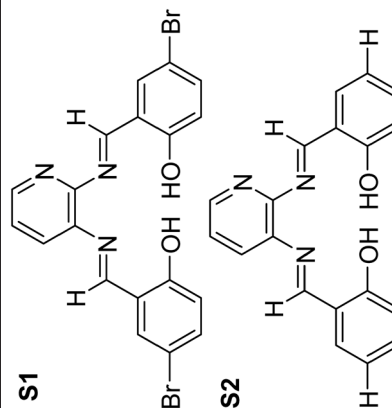


Table 1 (continued)

Small molecule	Chemical analogue	Mechanism of action	Inhibitory activity	Target	Safety profile	Ref.
S3		Pyrrole-carboxylic acid	N/A	IMP-1	No off-target toxicity	87
S4		Thiazolidine-carboxylic acid	Metal chelation; competitive inhibition	IMP-type-producing <i>E. coli</i> clinical isolate IMP-type-producing <i>E. coli</i> clinical isolate IMP-1 IMP-type-producing <i>K. pneumoniae</i>	CC ₅₀ value > 256 mg L ⁻¹ 85% viable HEK293 and L929 cell at >533 μM	88
S5		Thiosemicarbazone	Metal chelator; competitive inhibition; reversible inhibition	IMP-type-producing <i>Enterobacter</i> spp. IMP-type-producing <i>P. mirabilis</i> IMP-1 IMP-1 IMP-1-producing <i>E. coli</i>	IC ₅₀ value against Vero-E6 between 100 and 200 μM	89
S6		Picolinic acid	Metal chelator; non-competitive inhibition	IMP-1 IMP-1 IMP-4-producing clinical isolates	HC ₅₀ value > 1024 μg mL ⁻¹ <i>In vitro</i> showed NOAEL at 32 μg mL ⁻¹ <i>In vivo</i> showed NOAEL at 50 mg kg ⁻¹	90
S7		Picolinic acid	Metal chelator; non-competitive inhibition	IMP-1 IMP-1 IMP-4-producing clinical isolates	HC ₅₀ value > 1024 μg mL ⁻¹ <i>In vitro</i> showed NOAEL at 32 μg mL ⁻¹ <i>In vivo</i> showed NOAEL at 50 mg kg ⁻¹	90

Table 1 (continued)

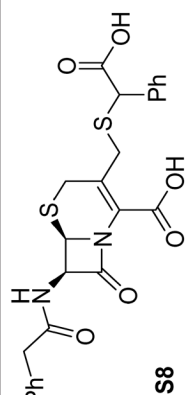
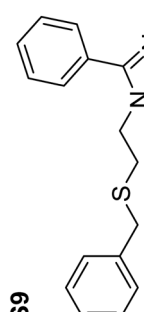
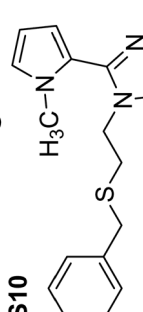
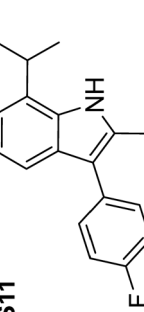
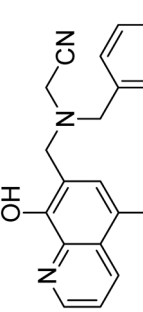
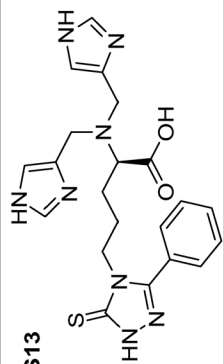
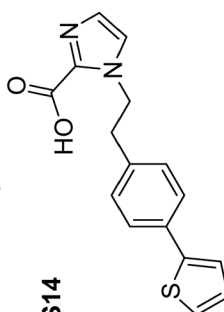
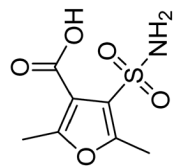
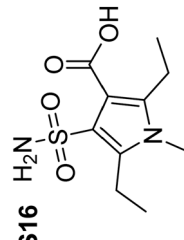
Small molecule	Chemical analogue	Mechanism of action	Inhibitory activity	Target	Safety profile	Ref.
	Cephalosporin-thiol	Metal chelator	IC ₅₀ value = 0.47 μ M IC ₅₀ value = 0.46 μ M MIC _{meropenem+s8} value = 1 μ g mL ⁻¹ MIC _{meropenem+s8} value = 0.063 μ g mL ⁻¹	IMP-1 IMP-28 IMP-1-producing <i>E. cloacae</i> IMP-28-producing <i>K. pneumonia</i>	N/A	91
	Thione	Metal chelator	K _i value = 19.6 μ M	IMP-1	IC ₅₀ value against HeLa cell = 152 μ M HC ₅₀ value > 500 μ M Potential off-target toxicity	62
	Thione	Metal chelator	K _i value = 9.04 μ M	IMP-1	IC ₅₀ value against HeLa cell >250 μ M HC ₅₀ value > 500 μ M	62
	Indole-carboxylic acid	Non-metal chelator	IC ₅₀ value = 0.140 μ M	IMP-1	No off-target toxicity	51
	Nitroxoline	Metal chelator	IC ₅₀ value of <3 μ M MIC _{imipenem+s12} value = 1 mg L ⁻¹ MIC _{imipenem+s12} value = 16 mg L ⁻¹	IMP-1 IMP-1-producing <i>P. aeruginosa</i> clinical isolate IMP-7-producing <i>P. aeruginosa</i> clinical isolate	IC ₅₀ value against HUVEC cell 45 μ g mL ⁻¹ May induce cell apoptosis	70

Table 1 (continued)

Small molecule	Chemical analogue	Mechanism of action	Inhibitory activity	Target	Safety profile	Ref.
S13 	Thiazolidine-carboxylic acid	Metal chelator; competitive inhibition	IC ₄₀ value = 100 μ M	IMP-1	Metabolically stable; IC ₅₀ value against HeLa cell >250 μ M; no off-target toxicity	92
S14 	Azole/imidazole-carboxylic acid	Metal chelator	IC ₅₀ value = 64.02 μ M	IMP-1	N/A	93
S15 	Sulfamoylfuran	Metal chelator; competitive inhibition	K _i value = 0.22 μ M FIC index value = 0.11 MIC _{meropenem} -S31 values reduced by 64-fold Rescued 90% infected murine model	IMP-1 IMP-1-producing engineered <i>E. coli</i> 19 IMP-1-producing <i>Enterobacteriaceae</i> clinical isolates IMP-1-producing <i>E. coli</i> clinical isolate	IC ₅₀ value against angiotensin-converting enzyme >1000 μ M; >50% viable HeLa cell at >1000 μ M; LD ₅₀ value intravenously on mice model = 246 mg kg ⁻¹ ; LD ₅₀ value intraperitoneally on mice model >1000 mg kg ⁻¹	94
S16 	Sulfamoylfuran	Metal chelator; competitive inhibition	K _i value = 0.26 μ M FIC index value = 0.02 MIC _{meropenem} -S32 values reduced by 128-fold Rescued 100% infected murine model	IMP-1 IMP-1-producing engineered <i>E. coli</i> 19 IMP-1-producing <i>Enterobacteriaceae</i> clinical isolates IMP-1-producing <i>E. coli</i> clinical isolate	>50% viable HeLa cell at >1000 μ M	94

Hint: NOAEL = no observed adverse effect level and NA = not available in report.

pneumonia was restored to moderate degrees. The compound exhibited organism-based antibacterial activity against the NDM-1-producing *Enterobacteriaceae*, with a minimum inhibitory concentration 50% (MIC₅₀) value of 32 μM . Interestingly, even at a high concentration (128 μM), this compound displayed an insignificant haemolytic effect, implying that the eukaryotic cell membrane was less affected,² supporting its systemic safety. In another report, a popular flavonol in fruits and vegetables called fisetin (**N2**) potently inhibited NDM-1 and NDM-9 with IC₅₀ values of 9.68 and 25.98 $\mu\text{g mL}^{-1}$, respectively. Its combination with meropenem against NDM-1-positive strains reduced the MIC values by a factor of 4–8 *in vitro* *E. coli* cells and restored the bactericidal activity of meropenem *in vivo* bacteria-infected mice model. The docking analysis revealed a strong interaction between key NDM-1 residues (Val 73, Met 248 and His 250) and fisetin, which contributed to the inhibitory effect. Through the formation of a ternary complex (meropenem-NDM-**N2**), which is categorized under a metal-chelating mechanism, **N2** inhibited NDM-1 non-competitively and reversibly. In addition, acute treatment (at 2000 mg kg^{-1} for 2 days) and chronic treatment (at 25 mg kg^{-1} for 9 months) of **N2** did not induce any significant toxicity,⁹⁵ indicating the safety of this active compound.

Similarly, withaferin A (**N3**) isolated from *Acnistus arborescens* and *Withania somnifera*, and a xanthone, mangiferin (**N4**) isolated from *Mangifera indica* showed theoretical binding to NDM-1 with a glide score of –5.12 and –9.12 kcal mol^{-1} upon docking, respectively. The phytochemicals also interacted with the Zn co-factor. The interesting theoretical findings were validated experimentally with IC₅₀ values of 24.03 μM for **N3** and 30.60 μM for **N4**, both indicating considerable inhibitory potentials. Their synergistic effects were observed when combined with imipenem against the carbapenem-resistant *A. baumannii*, suggesting augmentation/attenuation mechanisms of actions. Upon computational predictions of their pharmacokinetic properties, **N3** and **N4** displayed ideal parameters as promising candidates,⁹⁶ making them worthy of further assessment as potential antibiotics. Carnosic acid (**N5**), a terpenoid found in *Salvia officinalis* and *Rosmarinus officinalis*, inhibited NDM-1 in an enzyme inhibition assay quantitatively by IC₅₀ value = 27.07 μM . However, **N5** did not show strong inhibitory potential on bacterial growth on its own. Nevertheless, when combined with meropenem, it demonstrated a significant inhibitory effect on NDM-1-producing *E. coli* (the MIC value was reduced by 4-fold, from 16 to 4 $\mu\text{g mL}^{-1}$). The interesting synergy between the compound and meropenem was also revealed in the time-kill assay and checkerboard experiment, where **N5**-meropenem notably caused the *E. coli* population to drop to a negligible amount in just 4 h compared to other controls with a bacterial population maintained at $>10^5$ colony-forming unit (CFU)/mL. According to the docking analysis, **N5** can bind to residues such as Phe 46, Try 64, Leu 65, Asp 66, and Thr 94, implying an allosteric inhibitory mechanism to distort the

central NDM-1 site for enzymatic activity.⁹⁷ This further supported the potential of this phytochemical to preserve the antibiotic integrity of meropenem from NDM deactivation.

Among the inhibitors from synthetic routes, the aforementioned **S3** inhibited NDM-1 with an IC₅₀ value of 0.83 μM in addition to its IMP-1 inhibition. The combination therapy of **S3**-meropenem against two *E. coli* together with two *K. pneumonia* clinical isolates expressing NDM-1 resulted in impressive synergistic effects indicated by a significant decrease in MIC values of meropenem by 32–128-fold. Further experiments on resistant-strain bacteria-infected mice model (*in vivo*) also supported the synergistic efficacy, with a marked reduction in bacterial burden at the thigh tissue compared to meropenem therapy alone.⁸⁷ The shreds of experimental evidence cumulatively projected compound **S3** as a promising broad-spectrum inhibitor of MBL for further translational studies. Consistent with its inhibitory effects on IMP-1, **S4** also potently inhibited NDM-1 with a K_i value of 0.60 μM . The presence of **S4** reportedly prevented NDM-1 from the catalytic hydrolysis of imipenem *in vitro*, yielding an IC₅₀ value of 10 μM . Hydrophobic interactions between Trp 87, the main residue in NDM-1, and the inhibitor were observed, as well as the thiazolidine sulfate interacting with the conserved residue Trp 87,⁸⁸ supporting its interactive mechanism of broad-spectrum MBL inhibition. In addition to its interesting activity on IMP-1, **S5** also inhibited NDM-1 strongly with IC₅₀, IC₉₀, and K_i values of 0.021 μM , 0.2 μM , and 10.2 nM respectively. Although the combination of **S5** (at 4 $\mu\text{g mL}^{-1}$) with meropenem did not show a significant synergistic effect against *E. coli*-IMP-1. However, the application of the combined therapy on *E. coli*-NDM-1 reduced the MIC of meropenem from 128 to 8 $\mu\text{g mL}^{-1}$ (16-fold). **S5** (at 8 $\mu\text{g mL}^{-1}$)-meropenem also restored the carbapenem activity, as well as further reduced the MIC of meropenem by 256-fold, indicating a concentration-dependent antibiotic activity primarily targeting NDM-1. Moreover, **S5**-meropenem treatment significantly reduced the bacteria loads in mice models infected with a carbapenem-resistant *K. pneumonia* clinical isolate.⁸⁹ This comprehensive study promotes compound **S5** as a notable inhibitor of NDM-1 amenable for antibiotic development. Similarly, **S6** and **S7** also displayed excellent inhibitory effects against NDM-1, with IC₅₀ values of 1.6 and 1.69 μM and K_i values of 1.59 and 1.70 μM , respectively. According to time-kill kinetic assays, the population of NDM-1-carrying bacteria decreased dramatically (by $>10\,000$ -fold) after treatment with **S6**-meropenem and **S7**-meropenem combinations,⁹⁰ indicating attenuating effects.

The previously mentioned analogues of 1,2,4-triazole-3-thione with aryl sulfide with inhibitory effects on IMP-1, **S9** and **S10**, also inhibited NDM-1 effectively, as indicated by their K_i values of 22.3 and 28 μM , respectively. Equilibrium dialysis, metal analyses, and native mass spectrometry (MS) were used to validate the inhibitory mechanism, which was ternary complex formation between the inhibitors and NDM-1, a metal chelating mechanism. The addition of **S9** and

colistin to meropenem reduced the MIC value against a clinical isolate of NDM-1-producing *E. coli* by a factor of 8 (from 64 to 8 $\mu\text{g mL}^{-1}$), signifying their synergistic activity.⁶² Similarly, **S11** reportedly inhibited a wide range of MBL, notably, with an IC_{50} value of 5 nM against NDM-1. **S11** had no inherent antibacterial activity, but at $<15.6 \mu\text{M}$, it synergistically improved the effects of meropenem on an NDM-1-producing *E. coli* isolate, as indicated by the 4-fold reduction in MIC value from 64 to 16 $\mu\text{g mL}^{-1}$. The checkboard assay (fractional inhibitory concentration, FIC index value < 0.258) confirmed the synergy,⁵¹ making this compound promising for attenuating antibiotics. The nitroxoline derivative, **S12**, also had an inhibitory effect against NDM-1, with a concentration of 3 μM , inhibiting 68% of the enzyme. Furthermore, **S12** (at 32 mg L^{-1}) successfully restored meropenem activity against engineered NDM-1-producing *E. coli* (256-fold reduction in MIC from 128 to 0.5 mg L^{-1}) and clinically isolated NDM-1-producing *K. pneumonia* (64-fold reduction from 16 to 0.25 mg L^{-1}). The combinatory effect was also observed with other β -lactams such as piperacillin, cefuroxime, ceftazidime, and imipenem. In a clinical isolate of NDM-5-expressing *E. coli*, **S12**–imipenem reduced the MIC of imipenem by 8-fold (from 8 to 1 mg L^{-1}) and the bacterial population of NDM-1-producing *K. pneumonia* to a minimal extent (0.01%), as determined by the time-kill kinetic test.⁷⁰

An azetidinimine analogue, (*E*)-4-(2-(4-chlorophenyl)-4-((4-iodophenyl)imino)azetidin-1-yl)phenol (**S17**), showed a maximal inhibitory effect (100%) against NDM types (NDM-4, NDM-7, and NDM-9) and VIM type (VIM-1) at a dose of 10 μM . This enzymatic inhibition did not affect VIM-52, a variant of VIM-1 with a mutation at position 224 in the L3 loop. This provides basic evidence for the mechanism of action, specifically through the interaction between VIM-1 and NDM-1 at the L3 loop. **S17** is a non-covalent inhibitor with a K_i of 0.07 μM , which efficiently inhibits NDM-1. Also, **S17**–imipenem potentiates imipenem action and lowers the MIC against NDM-1-generating *E. coli*. Nonetheless, there are moderate cytotoxic effects indicated by an IC_{50} value of 19.9 μM against the MRC-5 cell line and 32.3 μM against the HCT-116 cell line.¹⁷ A derivative of phosphoramidate, (*R/S*)-(2-(methoxy(methyl(pyridin-2-ylmethyl)amino)phosphoryl)ethyl) ethanethioate (**S18**), inhibited NDM-1 quantitatively with an IC_{50} value of 356 μM . In combination, **S18**–meropenem neither showed a significant inhibitory effect on the *E. coli* strain expressing NDM-1 nor potential to restore meropenem activity. According to the NMR, docking, and chemical shift perturbation (CSP) results of a similar compound with phosphoramidate moiety, the main interaction was hydrophobic interactions, and the Trp 93 residue of loop 5 was the residual target; thus, it was located close to the catalytically important Zn. Likewise, **S18** demonstrated low cytotoxic value against the HeLa cell line (IC_{50} value $> 500 \mu\text{M}$). Ideally, this small-molecule inhibitor can serve as a template for a new effective standalone MBL inhibitor for future optimization.⁶⁴ A diaryl-substituted

thiosemicarbazone, (*E*)-4-(4-chlorophenyl)-1-(pyridin-2-ylmethylene)thiosemicarbazide (**S19**), reportedly showed strong inhibitory activity on the NDM-1 variant with an IC_{50} value of 0.038 μM . Further testing with a Zn-content determination assay revealed that **S19** deactivated the enzyme by removing Zn from the active site, a metal chelating mechanism. It demonstrated no significant bactericidal activity on its own, but when combined with meropenem against *E. coli* clinical isolates containing NDM-1, the MIC value reduced from 128 to 0.25 μM (512-fold), indicating the restoration of meropenem susceptibility. Furthermore, **S19**–meropenem treatment distinctly lowered the bacteria loads in a murine model infected with carbapenem-resistant *E. coli*, producing NDM-1. Interestingly, **S19** had a tolerable cytotoxic effect, as measured using the L929 cell line, in which it exhibited $>60\%$ viability following incubation at 50 μM .⁹⁸

Another study revealed that quinolinyl sulfonamides, specifically 4-chloro-*N*-(quinolin-8-yl)benzenesulfonamide (**S20**), exhibited high inhibitory potency against NDM-1, as indicated by its IC_{50} and K_i values of 0.02 μM and 5.9 nM, respectively. This compound was also demonstrated to inhibit NDM-1 competitively through a Lineweaver–Burk plot. Furthermore, according to ultraviolet-visible spectroscopy (UV-vis spectra) analysis, **S20** formed a complex with the Zn of the NDM-1 active site, suggesting a metal chelating mechanism. It also recovered the antibacterial action of meropenem on *E. coli*-NDM-1, as indicated by a reduction in MIC value from 128 to 8 $\mu\text{g mL}^{-1}$ (16-fold). The significant reduction in bacterial load in murine models infected with NDM-1-carrying *E. coli* clinical strain after using the **S20**–meropenem demonstrated the potential therapeutic applicability of the compound as an adjuvant. However, treatment with **S20** at 12.5 μM resulted in $<60\%$ viability of the MCF-7 cell line, indicating a non-toxic profile.⁹⁹ A derivative of pyrrolidine scaffold, ($\pm$)-*trans*-methyl 4-(4-(benzyloxy)-3-methoxyphenyl)-1-(2-(bis(pyridin-2-ylmethyl) amino)acetyl)pyrrolidine-3-carboxylate (**S21**), reportedly induced considerable NDM-1 inhibition, as revealed by its IC_{50} and K_i values of 51 and 4.6 μM , respectively. The addition of the Zn compound to the enzyme inhibition assay doubled the IC_{50} and K_i values, as well as comparative studies with EDTA suggested Zn displacement from the NDM-1 active site, a metal chelating mechanism as a plausible inhibitory pathway. Interestingly, **S21**–meropenem did not only restore meropenem activity but also prompted high synergistic activity against numerous NDM-1 positive CRE with FIC index values ranging from 0.01 to 0.25. **S21** had low cytotoxicity, with an IC_{50} value of greater than 128 μM against the HEK293 cell line,¹⁰⁰ indicating the potential of this compound as an effective and safe inhibitor of NDM-1 for further studies.

In addition, a benzoxazole-containing molecule, 2-(6-benzyloxy-1-hydroxynaphthalene-2-yl)benzoxazole-4-carboxylic acid (**S22**), inhibited NDM-1 with a determined IC_{50} value of 0.38 μM and a predicted binding energy value of $-9.99 \text{ kcal mol}^{-1}$. The docking results revealed that **S22** interacted with

all ten important NDM-1 amino acid residues, potentially reducing the enzyme affinity to β -lactams. Thus, **S22** had activity in restoring meropenem susceptibility against NDM-1-expressing *E. coli* strains. **S22** (at 25 μM)–meropenem also had a synergistic effect, lowering the MIC value from 64 to 16 $\mu\text{g mL}^{-1}$ (4-fold reduction). The small-molecule inhibitory action on MBL was suggested through UV-vis spectral analysis as ternary complex formation, a metal chelating mechanism, which is superior to non-specific metal removal action.⁸² (*E*)-*N'*-(2-Hydroxybenzylidene)-4-methylpiperazine-1-carbothiohydrazide (**S23**) is a derivative of thiosemicarbazones, which reportedly inhibited NDM-1 in a non-competitive manner (IC_{50} value = 0.0837 $\mu\text{g mL}^{-1}$ and K_i value = 0.44 $\mu\text{mol L}^{-1}$). Metal suppression experiments and molecular docking revealed that its interaction with Zn influences its inhibitory activity against NDM-1-harboring *K. pneumonia*, and **S23** is most likely to bind to the NDM-1 allosteric site (allosteric inhibitor). This compound also displayed potency in combination with meropenem against numerous clinical isolates carrying *bla*_{NDM-1}. For instance, **S23** (at 32 $\mu\text{g mL}^{-1}$)–meropenem facilitated meropenem antibacterial activity restoration (MIC_{90} value = 0.5 $\mu\text{g mL}^{-1}$). Based on the time-killing kinetics assay, this combination therapy resulted in a 3-fold decrease in the log population of *K. pneumonia*-NDM-1. Impressively, **S23** treatment on HeLa cell lines showed an IC_{50} value of >64 $\mu\text{g mL}^{-1}$, in the haemolytic assay, and it displayed haemolytic concentration 5%, HC_5 of >1000 mg mL^{-1} , and in an *in vivo* murine model, an NOAEL of <64 mg kg^{-1} was recorded, altogether indicating its good safety.¹⁰¹

A cephalosporin analogue, (6*R*,7*S*)-3-((benzo[*d*]thiazol-2-ylthio)methyl)-7-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (**S24**), was reported to show potent action against NDM-1, where the IC_{50} value was recorded as 0.13 μM . The IC_{50} values against NDM-3 (0.19 μM), NDM-4 (0.21 μM), NDM-12 (0.20 μM), and NDM-13 (0.16 μM), indicated that **S24** was effective on other NDM-type variants as well, showing its potential broad-spectrum activity. To exert inhibitory action, **S24** required the hydrolytic action of NDM-type. With a K_i value of 0.21 μM , the Dixon plot suggested competitive and reversible inhibitory mechanisms. According to the crystallographic structure analysis, NDM-1-mediated hydrolysis hypothetically produced a hydrolytic intermediate containing carboxylate and imine, which induced strong binding to Zn at the enzymatic site, *i.e.*, a metal chelating mechanism. Moreover, the presence of a hydrogen bond interaction between the inhibitor and Asp 124 resulted in the formation of a highly stable inhibitor–enzyme complex. Despite the low antibacterial activity of **S24** against *E. coli*-susceptible strains, **S24** (at 8 $\mu\text{g mL}^{-1}$)–meropenem efficaciously reduced the MIC of meropenem against the engineered *E. coli* strain (expressing *bla*_{NDM-1} gene) from 64 to 8 $\mu\text{g mL}^{-1}$ (8-fold difference). The high biocompatibility on mammalian cells was indicated after treating HEK 293 cell lines with the compound, where the NOAEL concentration was up to 100 μM .⁴⁶

Chen and colleagues reported the synthesis of a dithiocarbamate compound incorporating a copper (Cu) ion, SA09-Cu (**S25**), which had a novel inhibition mechanism for MBLs. **S25** not only inhibited NDM-1 effectively (with IC_{50} value = 0.096 μM), but it also synergized well with meropenem. The FIC index value determined when inhibitor–meropenem was used against engineered *E. coli*-harboring NDM-1 was 0.020, whereas the FIC index values determined when combination therapy was used against ten NDM-1-producing clinical isolates ranged from 0.031 to 0.375, indicating a significant improvement. The time-kill assay revealed an apparent lowered population of NDM-1-producing *E. coli* clinical isolates upon **S25**–meropenem treatment, showcasing the synergist effect. According to the Lineweaver–Burk plot and electron paramagnetic resonance (EPR) spectroscopy, **S25** bound non-competitively to NDM-1, and more importantly showed potential to oxidize the Zn(II) thiolate of the enzyme, rendering NDM-1 deactivation. Strikingly, the cytotoxic assay indicated that **S25** (at 40 $\mu\text{g mL}^{-1}$) could still maintain >60% viability in Vero E6 and L929 cell lines, whereas the *in vivo* mouse model evidenced low acute toxicity (lethal dose 50%, LD_{50} value > 60/6 mg kg^{-1}). Cumulatively, **S25** possesses a relatively strong antibiotic effect and good safety profile.⁶⁵ 4-3-([1,1'-Biphenyl]-3-yl)propyl)benzene-1,3-diol (**S26**), a 1,2,4-triazole-3-thione analogue, exhibited high inhibitory potency against NDM-1, with a K_i value of 14.3 μM . **S26** was able to moderately potentiate meropenem against a clinical isolate of NDM-1-producing *E. coli* by a factor of two (MIC value reduced from 64 to 32 $\mu\text{g mL}^{-1}$). **S26** had a minor off-target effect (IC_{50} value against angiotensin-converting enzyme <100 μM) and was non-cytotoxic (IC_{50} value against HeLa cells >250 μM).¹⁰² Jackson *et al.* presented 2-((((6*R*,7*R*)-2-carboxy-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl)thio)pyridine 1-oxide (**S27**), a small molecule with a cephalosporin core, which converted into a metal chelator upon enzyme hydrolysis. **S27** restored the meropenem antibacterial effect in both NDM-1-producing engineered and clinical *E. coli* strains, in addition to effectively inhibiting purified NDM-1 (IC_{50} value of 7.6 μM). The co-treatment of **S27** at 20 μM reduced the MIC values of meropenem from more than 61 to 3.81 $\mu\text{g mL}^{-1}$ (>16-fold), and higher concentrations of **S27** were thought to restore meropenem activity. Through equilibrium dialysis experiments and UV-visible spectra, **S27** was discovered to inhibit NDM-1 not through Zn removal but through the formation of ternary complexes (a metal chelating mechanism).¹⁰³

3-((3-Mercaptopropyl)thio)-1-phenylpyrrolidin-2-one (**S28**), an *N*-phenyl derivative, effectively inhibited NDM-1 (IC_{50} value of 0.3 μM). Although **S28** (at 512 μM) has no intrinsic antibacterial properties, it restored imipenem activity in a dose-dependent manner against an NDM-1-producing engineered *E. coli* strain. **S28** at the highest concentration tested (128 $\mu\text{g mL}^{-1}$) reduced the MIC of imipenem from 128 to 16 $\mu\text{g mL}^{-1}$ (8-fold). Docking predicted the binding mode of **S28**, in which a free thiol group coordinates between the Zn of the enzymatic active site (a metal chelating

mechanism), displacing water and halting β -lactam hydrolysis. **S28** also could bind to and directly inhibit MBLs.¹⁰⁴ **S13** was reported to have a strong inhibitory effect on NDM-type enzyme (K_i value against NDM-1 of 0.03 μM), despite having a modest impact on IMP-type enzyme. Furthermore, co-treatment with **S13** at a concentration of 32 $\mu\text{g mL}^{-1}$ could potentially revive the action of meropenem, given that the MIC of meropenem against an ultra-resistant *E. coli* clinical strain that produces NDM-1 was reduced from 64 to 0.06 $\mu\text{g mL}^{-1}$ (>1000-fold).⁹² The previously mentioned **S14** also inhibited the NDM-type enzyme, although with a milder effect (IC_{50} value of 126.2 μM).⁹³

6-((Bis(pyridin-2-ylmethyl)amino)methyl)-*N*-methyl-*N*-((2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl)nicotinamide (**S29**), which was synthesized by Samuelsen and colleagues, had no intrinsic antibacterial effect up to 500 μM , but the **S29**-meropenem combination reduced the MIC values of meropenem against 234 MBL producing clinical *Enterobacterales* strains by 128-fold (from 64 to 0.5 $\mu\text{g mL}^{-1}$). Surprisingly, **S29** exposure, whether single or serially passaged, was unlikely to result in resistant mutation. Furthermore, compared to the negative control, **S29**-meropenem treatment significantly reduced the bacteria loads in murine models infected with meropenem-resistant *K. pneumonia* that produce NDM-1. The mechanism of action of **S29** is through the removal of Zn from the enzymatic active site (a metal chelating mechanism), and the inhibition was shown to be irreversible. Also, **S29** demonstrated low toxicity *in vitro* in the HepG2 cell line (IC_{50} value > 100 μM) and *in vivo* in the murine model (NOAEL < 252 $\text{mg kg}^{-1}/7$ weeks), high selectivity for bacterial MBLs, and low plasma protein binding.¹⁰⁵ Although **S15** had a K_i value of 9.82 μM versus NDM-1, **S16** had a K_i value of 0.84 μM versus NDM-1. When **S15** or **S16** were combined with meropenem NDM-1-expressing *E. coli* strain, the MIC of meropenem was reduced by 4-fold (from 64 to 16 $\mu\text{g mL}^{-1}$) and 256-fold (from 64 to 0.25 $\mu\text{g mL}^{-1}$), respectively. At a concentration of 4 $\mu\text{g mL}^{-1}$, **S15** reduced the MIC value of meropenem by only 2-fold, whereas **S16** reduced it by 32-fold against 14 NDM-type *Enterobacteriaceae* clinical isolates. After the administration of meropenem-**S16** (10 mg kg^{-1}) combination therapy in the murine model (infected with NDM-1/VIM-1-carrying *K. pneumonia* clinical strain), 100% of the murine model survived.⁹⁴ A summary of the activity data including mechanisms of actions and safety profiles of selected potent small-molecule inhibitors of NDM curated from the recent literature is presented in Table 2.

Inhibitors of Verona-integron metallo- β -lactamase

Due to its interesting potential against IMP-1 and NDM-1, **S3** displayed efficacy for inhibiting VIM-types quantitatively with an IC_{50} value against VIM-2 of 32.37 μM , suggesting a broad spectrum of MBL inhibitory properties. With meropenem, its MIC_{50} values against two VIM-1-producing *K. pneumonia* clinical isolates were reduced from 1 to 0.06 mg L^{-1} (16-fold)

Table 2 Summary of selected potent small-molecule inhibitors of NDM, their targets, mechanisms of action and toxicity profiles

Small molecule	Chemical analogue	Mechanism of action	Activity	Target	Safety profile	Ref.
N1	Ketone	Metal chelator	IC_{50} value = 12.1 μM K_d value = 11.8 μM Synergism with meropenem and imipenem MIC_{50} value = 32 μM	NDM-1 NDM-1 NDM-1-producing <i>E. coli</i> and <i>K. pneumoniae</i> NDM-1-producing <i>Enterobacteriaceae</i>	Insignificant haemolytic effect at 128 μM	2
N2	Flavonoid	Metal chelator; non-competitive inhibition; reversible	IC_{50} value = 9.68 $\mu\text{g mL}^{-1}$ IC_{50} value = 25.98 $\mu\text{g mL}^{-1}$ $\text{MIC}_{\text{meropenem+N2}}$ values reduced by 4- to 8-fold Restored meropenem action <i>in vivo</i>	NDM-1 NDM-9 NDM-1-producing strains	NOAEL at 2000 mg kg^{-1} for 2 days and at 25 mg kg^{-1} for 9 months	95

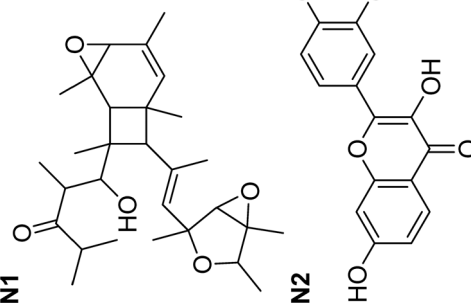


Table 2 (continued)

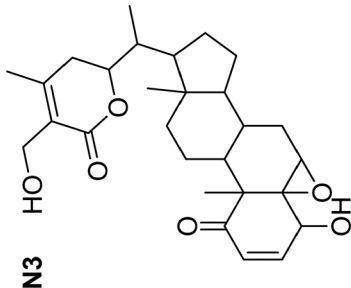
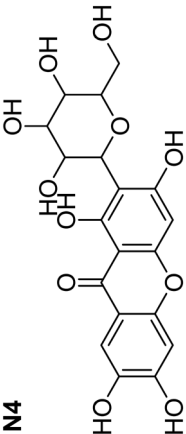
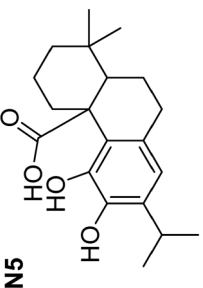
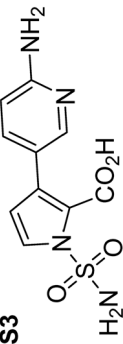
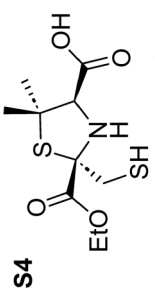
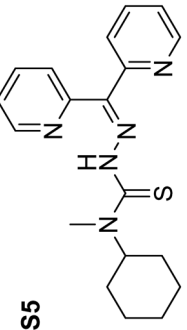
Small molecule	Chemical analogue	Mechanism of action	Activity	Target	Safety profile	Ref.
N3 	Oxiren-one	Augmentation effect	IC ₅₀ value = 24.03 μM Synergism with imipenem	NDM-1 Carbapenem-resistant <i>A. baumannii</i>	N/A	96
N4 	Flavonoid	Augmentation effect	IC ₅₀ value = 30.60 μM Synergism with imipenem	NDM-1 Carbapenem-resistant <i>A. baumannii</i>	N/A	96
N5 	Phenanthrene-carboxylic acid	Allosteric inhibitor	IC ₅₀ value = 27.07 μM MIC _{meropenem+N5} value = 4 μg mL ⁻¹	NDM-1 NDM-1-producing <i>E. coli</i>	N/A	97
S3 	Pyrrole-carboxylic acid	N/A	IC ₅₀ value = 0.83 μM MIC _{meropenem+S3} values reduced by 32- to 128-fold <i>In vivo</i> showed synergism with meropenem	NDM-1 NDM-1-producing <i>E. coli</i> and <i>K. pneumonia</i> clinical isolates —	No off-target toxicity CC ₅₀ value > 256 mg L ⁻¹	87
S4 	Thiazolidine-carboxylic acid	Metal chelator; competitive inhibition	K _i value = 0.60 μM IC ₅₀ value = 10 μM	NDM-1 NDM-1	85% viable HEK293 and L929 cells at > 533 μM	88
S5 	Thiosemicarbazone	Metal chelator; competitive inhibition; reversible	IC ₅₀ value = 0.021 μM IC ₉₀ value = 0.2 μM K _i value = 10.2 nM Restored carbapenem action <i>in vitro</i> and <i>in vivo</i>	NDM-1 NDM-1 NDM-1 Carbapenem-resistant <i>K. pneumonia</i> clinical isolate	IC ₅₀ value against Vero-E6 between 100 and 200 μM	89

Table 2 (continued)

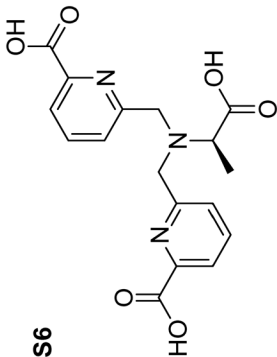
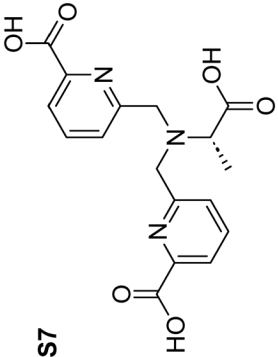
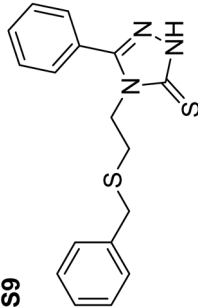
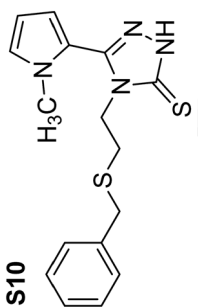
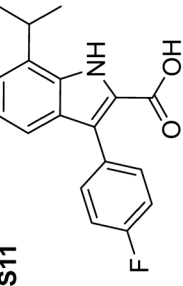
Small molecule	Chemical analogue	Mechanism of action	Activity	Target	Safety profile	Ref.
	Picolinic acid	Metal chelator; non-competitive inhibition	IC ₅₀ value = 1.6 μM K _i value = 1.69 μM Meropenem + S6 reduced bacteria population by >10 000-fold	NDM-1 NDM-1 NDM-1-producing bacteria	HC ₅₀ value > 1024 μg mL ⁻¹ <i>In vitro</i> showed NOAEL at 32 μg mL ⁻¹ <i>In vivo</i> showed NOAEL at 50 mg kg ⁻¹	90
	Picolinic acid	Metal chelator; non-competitive inhibition	IC ₅₀ value = 1.69 μM K _i value = 1.70 μM Meropenem + S7 reduced bacteria population by >10 000-fold	NDM-1 NDM-1 NDM-1-producing bacteria	HC ₅₀ value > 1024 μg mL ⁻¹ <i>In vitro</i> showed NOAEL at 32 μg mL ⁻¹ <i>In vivo</i> showed NOAEL at 50 mg kg ⁻¹	90
	Thione	Metal chelator	K _i value = 22.3 μM MIC _{meropenem+colistin+S9} value = 8 μg mL ⁻¹	NDM-1 NDM-1-producing <i>E. coli</i> clinical isolate	IC ₅₀ value against HeLa cell = 152 μM HC ₅₀ value > 500 μM Potential off-target toxicity	62
	Thione	Metal chelator	K _i value = 28 μM	NDM-1	IC ₅₀ value against HeLa cell >250 μM HC ₅₀ value > 500 μM	62
	Indole-carboxylic acid	Non-metal chelator	IC ₅₀ value = 5 nM Restore meropenem at <15.6 μM MIC _{meropenem+S11} value = 16 μg mL ⁻¹ FIC index value = 0.258	NDM-1 NDM-1-producing <i>E. coli</i> isolate NDM-producing strain NDM-producing strain	No off-target toxicity	51

Table 2 (continued)

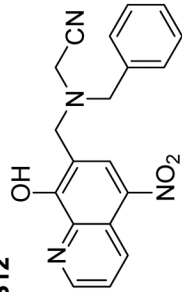
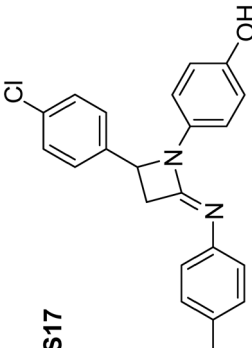
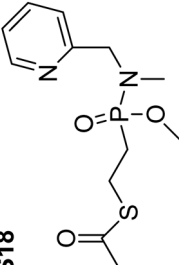
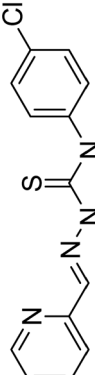
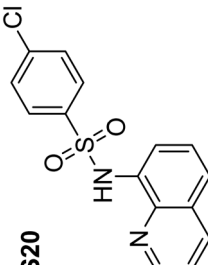
Small molecule	Chemical analogue	Mechanism of action	Activity	Target	Safety profile	Ref.
S12 	Nitroxoline	Metal chelator	IC ₅₀ value of <3 μM MIC _{meropenem+S12} value = 0.5 mg L ⁻¹ MIC _{meropenem+S12} value = 0.25 mg L ⁻¹ MIC _{imipenem+S12} value = 1 mg L ⁻¹ Imipenem + S12 reduced bacteria population >99.9% Synergism with piperacillin, cefuroxime, and cefazidime, and imipenem IC ₁₀₀ value < 10 μM	NDM-1 NDM-1-producing engineered <i>E. coli</i> strain NDM-1-producing <i>K. pneumoniae</i> clinical isolate NDM-5-producing <i>E. coli</i> clinical isolate NDM-1-producing <i>K. pneumoniae</i> Multiple NDM-type-producing clinical isolates	IC ₅₀ value against HUVBC cell 45 μg mL ⁻¹ May induce cell apoptosis	70
S17 	Azetidinimine	Binding to L3 loop (residue 224) of MBLs; non-covalent inhibition	<i>K_i</i> value = 0.07 μM Restored imipenem action <i>in vitro</i>	NDM-4, NDM-7, NDM-9 NDM-1 NDM-1-producing <i>E. coli</i>	IC ₅₀ value against MRC-5 cell = 17.9 μM IC ₅₀ value against HCT-116 cell 32.3 μM	17
S18 	Phosphoramidate	Binding to loop 5 (93 residues)	IC ₅₀ value = 356 μM	NDM-1	IC ₅₀ value against HeLa cell >500 μM	64
S19 	Thiosemicarbazone	Metal chelator	IC ₅₀ value = 0.038 μM MIC _{meropenem+S15} value = 0.25 μM Restored action of meropenem <i>in vitro</i> and <i>in vivo</i>	NDM-1 NDM-1-producing <i>E. coli</i> clinical isolate —	>60% viable L929 cell at 50 μM	98
S20 	Sulfonamide	Metal chelator; competitive inhibition	IC ₅₀ value = 0.02 μM <i>K_i</i> value = 5.9 μM MIC _{meropenem+S16} value = 8 μg mL ⁻¹ Restored action of meropenem <i>in vitro</i> and <i>in vivo</i>	NDM-1 NDM-1 NDM-1-producing <i>E. coli</i> clinical isolate NDM-1-producing <i>E. coli</i> clinical isolate	<60% viable MCF-7 cell at 12.5 μM	99

Table 2 (continued)

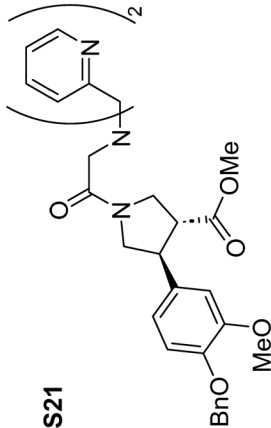
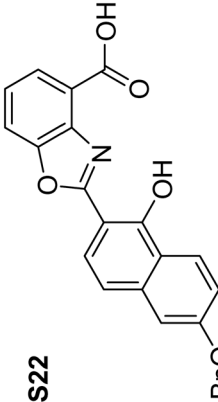
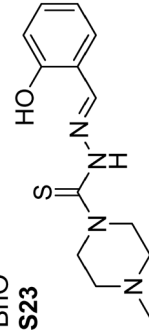
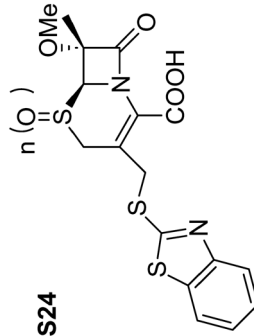
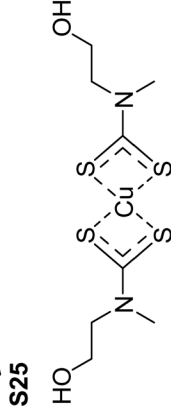
Small molecule	Chemical analogue	Mechanism of action	Activity	Target	Safety profile	Ref.
	Pyrrolidine-carboxylate	Metal chelator	IC ₅₀ value = 51 μM K _i value = 4.6 μM Synergism with meropenem FIC index value = 0.01 to 0.25	NDM-1 NDM-1 NDM-1-producing CRE NDM-1-producing CRE	IC ₅₀ value against HEK293 cell >128 μM	100
	Azole/imidazole-carboxylic acid	Metal chelator	IC ₅₀ value = 0.38 μM Restored meropenem action MIC _{meropenem+S18} value = 16 μg mL ⁻¹	NDM-1 NDM-1-producing <i>E. coli</i> NDM-1-producing <i>E. coli</i>	N/A	82
	Hydrazide	Allosteric inhibition	IC ₅₀ value = 0.0837 μg mL ⁻¹ K _i value = 0.44 μmol L ⁻¹ MIC ₉₀ of meropenem+S19 value = 0.5 μg mL ⁻¹ Restored meropenem action	NDM-1 NDM-1 Multiple NDM-1-producing clinical isolates NDM-1-producing <i>K. pneumoniae</i>	IC ₅₀ value against HeLa cell >64 μg mL ⁻¹ HC ₅ value > 1000 mg mL ⁻¹ <i>In vivo</i> showed NOAEL at <64 mg kg ⁻¹	101
	Cephalosporin	Metal chelation; competitive inhibition; reversible inhibition	IC ₅₀ value = 0.13 μM K _i value = 0.21 μM IC ₅₀ value = 0.19 μM IC ₅₀ value = 0.21 μM IC ₅₀ value = 0.20 μM IC ₅₀ value = 0.16 μM MIC _{meropenem+S20} value = 8 μg mL ⁻¹	NDM-1 NDM-1 NDM-3 NDM-4 NDM-12 NDM-13 NDM-1-producing engineered <i>E. coli</i>	<i>In vitro</i> showed NOAEL at 100 μM	46
	Copper-dithiocarbamate	Oxidative effect on Zn(II) thiolate to inactive MBL; non-competitive inhibition	IC ₅₀ value = 0.096 μM FIC index value = 0.02 FIC index value ranging between 0.031 to 0.375	NDM-1 NDM-1-producing engineered <i>E. coli</i> Multiple NDM-1-producing <i>E. coli</i> clinical isolates	>60% viable Vero E6 and L929 cells at 40 μg mL ⁻¹ LD ₅₀ value on mice model >60/6 mg kg ⁻¹	65
	Phenol	Metal chelator	K _i value = 14.3 μM Restored meropenem action	NDM-1 NDM-1-producing <i>E. coli</i> clinical isolate	IC ₅₀ value against angiotensin-converting enzyme <100 μM; IC ₅₀ value against	102

Table 2 (continued)

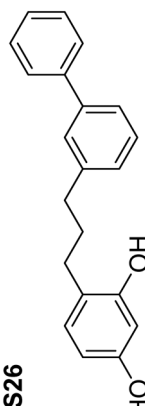
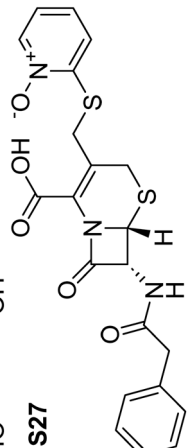
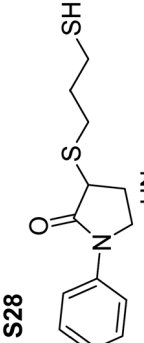
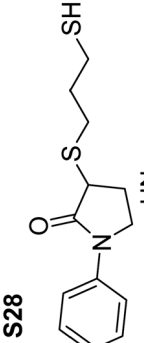
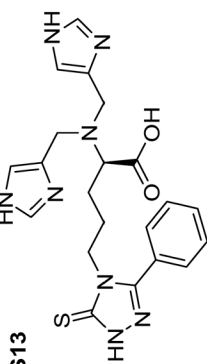
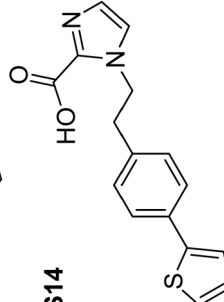
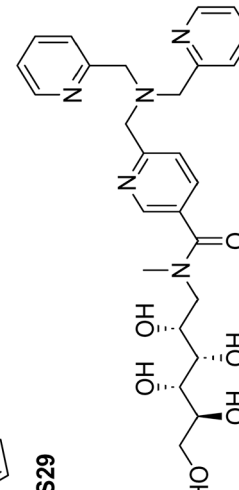
Small molecule	Chemical analogue	Mechanism of action	Activity	Target	Safety profile	Ref.
S26			$MIC_{meropenem+S26} = 32 \mu\text{g mL}^{-1}$	NDM-1-producing <i>E. coli</i> clinical isolate	HeLa cell $>250 \mu\text{M}$	
S27		Pyridine oxide	IC_{50} value = $7.6 \mu\text{M}$ Restored meropenem action	NDM-1 NDM-1-producing <i>E. coli</i> clinical isolate and engineered strain	N/A	103
S28			$MIC_{meropenem+S26} = 3.81 \mu\text{g mL}^{-1}$	NDM-1-producing <i>E. coli</i> clinical isolate and engineered strain		
S28		Pyrrolidinone	IC_{50} value = $0.3 \mu\text{M}$ Restored imipenem action	NDM-1 NDM-1-producing engineered <i>E. coli</i>	N/A	104
S13		Thiazolidine-carboxylic acid	K_i value = $0.03 \mu\text{M}$ Restored meropenem action $MIC_{meropenem+S28} = 0.06 \mu\text{g mL}^{-1}$	NDM-1 NDM-1-producing <i>E. coli</i> clinical isolate NDM-1-producing <i>E. coli</i> clinical isolate	Metabolically stable; IC_{50} value against HeLa cell $>250 \mu\text{M}$; no off-target toxicity	92
S14		Azole/imidazole-carboxylic acid	IC_{50} value = $126.2 \mu\text{M}$	NDM-1	N/A	93
S29		Nicotinamide	$MIC_{meropenem+S30}$ values reduced by 128-fold <i>In vivo</i> showed synergism with meropenem	234 NDM-1-producing clinical <i>Enterobacteriales</i> NDM-1-producing <i>K. pneumonia</i>	No risk of resistant mutation; IC_{50} value against HepG2 cell $>100 \mu\text{M}$; NOAEL at 252 mg kg^{-1} for 7 weeks; low plasma protein binding	105

Table 2 (continued)

Small molecule	Chemical analogue	Mechanism of action	Activity	Target	Safety profile	Ref.
S15	Sulfamoylfuran	Metal chelator; competitive inhibition	K_i value = 9.81 μM $\text{MIC}_{\text{meropenem}+\text{S15}}$ value = 16 $\mu\text{g mL}^{-1}$ $\text{MIC}_{\text{meropenem}+\text{S15}}$ values reduced by 2-fold	NDM-1 NDM-1-producing <i>E. coli</i> 14 NDM-1-producing <i>Enterobacteriaceae</i> clinical isolates	IC_{50} value against angiotensin-converting enzyme >1000 μM ; >50% viable HeLa cell at >1000 μM ; LD_{50} value on mice model intravenously = 246 mg kg^{-1} ; LD_{50} value on mice model intraperitoneally >1000 mg kg^{-1}	94
S16	Sulfamoylfuran	Metal chelator; competitive inhibition	K_i value = 0.84 μM $\text{MIC}_{\text{meropenem}+\text{S16}}$ value = 0.25 $\mu\text{g mL}^{-1}$ $\text{MIC}_{\text{meropenem}+\text{S16}}$ values reduced by 32-fold Rescued 100% infected murine model	NDM-1 NDM-1-producing <i>E. coli</i> 14 NDM-1-producing <i>Enterobacteriaceae</i> clinical isolates NDM-1/VIM-1-producing <i>K. pneumoniae</i> clinical isolate	>50% viable HeLa cell at >1000 μM	94

Hint: NOAEL = no-observed-adverse-effect level and N/A = not available in report.

and 0.5 to 0.06 mg L^{-1} (8-fold), indicating a synergistic effect.⁸⁷ Similar to its inhibition properties on IMP-1 and NDM-1, **S4** also effectively inhibited VIM-2 with a K_i of 1.9 μM . The promising inhibitor has a robust hydrophobic interaction with Trp87 in VIM-2, as observed from its structural analysis.⁸⁸ Furthermore, the idea of **S5** being a broad-spectrum MBL inhibitor was confirmed by its potent inhibitory action against VIM-2, exhibiting IC_{50} and IC_{90} values of 0.11 and 1 μM , respectively. **S5** (at 4 $\mu\text{g mL}^{-1}$)–meropenem reportedly had a fair synergistic effect against *E. coli*-VIM-2, where the MIC value was reduced from 64 to 8 $\mu\text{g mL}^{-1}$ (8-fold), whereas **S5** (at 8 $\mu\text{g mL}^{-1}$)–meropenem successfully reduced the MIC by 32-fold,⁸⁹ suggesting a concentration-dependent synergistic effect. Broad-spectrum inhibitory actions against multiple MBLs were also evidently observed for **S6** and **S7**, where they effectively hampered VIM-2 activity in addition to their potent inhibition of IMP-1 and NDM-1. The synthetic compounds **S6** and **S7** exhibited IC_{50} values of 4.40 and 4.17 μM , as well as K_i values of 6.16 and 5.83 μM against VIM-2, respectively. The time-kill kinetic assay findings revealed a significant reduction in the population of VIM-1-producing clinical isolates to a negligible detection amount (10^2 CFU mL^{-1}) with **S6**–meropenem and **S7**–meropenem,⁹⁰ supporting their synergistic actions.

Following the reports of 1,2,4-triazole-3-thione compounds with aryl sulfide, **S9** and **S10** for IMP-1 and NDM-1 inhibition, were synthesized by Legru and co-workers, the VIM types can also be the target of the inhibitors, respectively. For instance, **S9** had potent inhibitory actions against VIM-1 (K_i values = 0.21 μM), VIM-2 (K_i values = 0.24 μM), and VIM-4 (K_i values = 0.21 μM). Similar potency was observed for **S10** against VIM-1 (K_i values = 0.23 μM), VIM-2 (K_i values = 0.34 μM), and VIM-4 (K_i values = 0.25 μM). In combination, **S9**–meropenem and **S10**–meropenem potentiated meropenem against two VIM-1- and VIM-4-carrying *K. pneumoniae* clinical isolates effectively. For the VIM-1-carrying strain, the MIC values decreased from 16 to 2 $\mu\text{g mL}^{-1}$ (8-fold reduction) and 4 $\mu\text{g mL}^{-1}$ (4-fold reduction), while for the VIM-4-carrying strain, the MIC values decreased from 16 to 2 $\mu\text{g mL}^{-1}$ (8-fold reduction) and 1 $\mu\text{g mL}^{-1}$ (16-fold reduction) by the respective therapy. The FIC index value of 0.347 for **S10**–meropenem in the checkerboard assay validated this synergism.⁶² As another promising broad-spectrum MBL inhibitor, **S12** (at 3 μM) exclusively inhibited the VIM-1 enzyme by 98% in addition to its interesting actions on IMP-1 and NDM-1. It also displayed a combinatory effect with imipenem indicated by a 64-fold reduction in MIC value from 32 to 0.5 mg L^{-1} against a VIM-1-expressing transgenic *E. coli*. When the **S12**–imipenem synergy action was tested using a time-kill kinetic assay, they exerted up to 99.9% antibacterial effect against VIM-1-producing *E. coli*,⁷⁰ validating its potential for attenuating the antibacterial activity of imipenem.

A recent study on 1*H*-imidazole-2-carboxylic acid, Yan *et al.* showed that 1-(pyridin-4-ylmethyl)-1*H*-imidazole-2-

carboxylic acid (**S30**) has strong VIM-2 inhibitory properties, exhibiting an IC_{50} value of $0.56 \mu M$. According to the crystallographic analysis, **S30** interacted with VIM-type flexible active site loops (L3 and L10) to allow coordination with Zn in a metal chelating mechanism. However, the inhibitory activities against some other variants, VIM-1 and VIM-5, were deemed moderate, with IC_{50} values of 29.50 and $5.78 \mu M$, respectively. The MIC of meropenem was reduced by **S30** (at $10 \mu g mL^{-1}$) against three engineered *E. coli* strains containing VIM-2 from 16 to $0.25 \mu g mL^{-1}$ (64-fold), 16 to $0.25 \mu g mL^{-1}$ (64-fold), and 32 to $1 \mu g mL^{-1}$ (32-fold), respectively. The synergy was evidenced using the checkerboard assay with an FIC value of 0.05 . Transmission electron microscopy (TEM) revealed that **S30** successfully penetrated the cell and inhibited VIM-2. Further toxicological evaluation *in vivo* confirmed good pharmacokinetic parameters and a safe profile, with an NOAEL of $2000 mg kg^{-1}$,¹⁰⁶ supporting **S30** as a promising MBL inhibitor for further assessment. Verdirosa and co-researchers reported that 2-(2-(3-([1,1'-biphenyl]-3-yl)-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)ethyl)benzoic acid (**S31**), derived from 1,2,4-triazole-3-thione with *m*-biphenyl moiety, had potent inhibitory action against VIM types, with IC_{50} values of 1.61 , 0.08 , and $2.1 \mu M$ against VIM-1, VIM-2, and VIM-4, respectively. Besides, its K_i value against VIM-2 was $38 nM$. Crystallographic analysis of the complex formed by VIM-2 and this compound, which contains a triazole-thione core, revealed an interaction with Zn ions at the enzymatic binding site, indicating a metal chelating mechanism. The thermodynamic parameters indicated that **S31** was specific and selective in terms of VIM inhibition. Further, **S31**-meropenem showed effective synergy against two clinically isolated *K. pneumonia* expressing *bla*_{VIM-1} or *bla*_{VIM-4}, where the MIC value of meropenem was reduced from 16 to $2 \mu g mL^{-1}$ (8-fold) and 16 to $1 \mu g mL^{-1}$ (16-fold), respectively. Finally, a cell-based assay using the HeLa cell line demonstrated that **S31** (at $250 \mu M$) did not cause cell lysis, supporting its ideal safety.¹⁹

S26 also inhibited NDM-type and VIM-type enzymes in a micromolar range (IC_{50} value against VIM-1 = $0.41 \mu M$; IC_{50} value against VIM-2 = $1.3 \mu M$; and IC_{50} value against VIM-4 = $0.82 \mu M$). In combination with meropenem, **S26** significantly reduced the MIC values from 16 to $4 \mu g mL^{-1}$ (8-fold) and from 16 to $4 \mu g mL^{-1}$ (4-fold) against *K. pneumonia* clinical isolates harbouring VIM-1 and VIM-4, respectively. The authors used molecular modelling to show that the 1,2,4-triazole-3-thione core could coordinate with active site Zn ions of MBL (a metal chelating mechanism) and displaced the catalytic hydroxide anion.¹⁰² Besides inhibiting NDM-1, **S28** can also potently inhibit VIM-1 (IC_{50} value of $0.02 \mu M$ and K_d value < $0.88 \mu M$). In a dose-dependent manner, the MIC value of imipenem significantly decreased against the engineered strain of *E. coli* carrying the VIM-1, dropping from 32 to

$1 \mu g mL^{-1}$ (32-fold) at an **S28** concentration of $128 \mu g mL^{-1}$.¹⁰⁴

The sub-micromolar inhibitory range of **S13** also extended to VIM-type enzymes where the inhibitor was reported with K_i values of 0.15 , 0.41 and $0.20 \mu M$ against VIM-1, VIM-2 and VIM-4, respectively. It demonstrated a potentiation on meropenem against multiple VIM-type-carrying clinical strains, with the MIC values for *K. pneumonia* expressing VIM-1 and VIM-4 being reduced by more than 500-fold (from 16 to $0.03 \mu g mL^{-1}$) and *P. aeruginosa* expressing VIM-2 reduced by 16-fold (from 128 to $8 \mu g mL^{-1}$). The checkerboard assay was used to confirm the synergism of **S13**-meropenem, and an FIC index value of 0.34 was obtained.⁹² **S14** was more effective against VIMs, including VIM-1 (IC_{50} value = $3.98 \mu M$), VIM-2 (IC_{50} value = $0.018 \mu M$), and VIM-5 (IC_{50} value = $0.018 \mu M$). At a concentration of $10 \mu g mL^{-1}$, **S14** showed synergy *in vitro*, decreasing the MIC of meropenem against two VIM-2-harboured *E. coli* strains by more than 64-fold (from 16 to $\leq 0.25 \mu g mL^{-1}$). It was predicted using the docking method that this molecule coordinates to interact with Zn (a metal chelating mechanism) and interact with MBL residue, which is important for the hydrolysis of β -lactams. The authors hypothesised that its potency was due to its small size, allowing easy penetration through bacterial membranes.⁹³

The inhibitor of NDM with the sulfamoyl heteroarylcarboxylic acid skeleton, **S15**, inhibited VIM-2 competitively with a K_i value of $2.81 \mu M$ and showed synergism with meropenem against VIM-2-producing *E. coli* strains, as indicated by the FIC index value of 0.31 . **S16** also inhibited VIM-2 with improved potency (K_i = $0.02 \mu M$), and the combination of **S16** and ceftazidime significantly reduced the MIC value of ceftazidime against a VIM-2-producing *E. coli* strain from 16 to $1 \mu g mL^{-1}$ (16-fold). When tested against NDM-1/VIM-1-producing *K. pneumonia* strains, the checkerboard assay revealed synergism with meropenem, with **S15** and **S16** obtaining FIC index values of 0.19 and 0.05 , respectively.⁹⁴

The [1,2,4]triazole derivative, 3-(4-bromophenyl)-6,7-dihydro-5H-[1,2,4]triazolo[3,4-*b*][1,3]thiazine (**S32**) synthesised by Yuan *et al.* inhibited VIM-2, with an IC_{50} value of $38.36 \mu M$, but barely inhibited both VIM-1 and VIM-5, as indicated by its 23% and 33% enzyme inhibition at a concentration of $100 \mu M$, respectively. Further selectivity analysis confirmed its selectivity against VIM-2 MBL. Using molecular docking, **S32** was predicted to bind to Zn at the active binding site of VIM-2 (a metal chelating mechanism), as well as a critical residue for substrate recognition (Arg 228) and the flexible L1 loop.¹⁰⁷ Also, 2-((5-(2-hydroxyphenyl)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-(trifluoromethoxy)phenyl)acetamide (**S33**) with a thiazolethioacetamide skeleton was discovered to have a potent inhibitory effect on VIM-2 (IC_{50} value of $2.2 \mu M$). However, **S33** could not synergize with cefazolin and restore its activity against *E. coli* expressing VIM-2. To

Table 3 Summary of selected potent small-molecule inhibitors of VIM, their targets, mechanisms of action and toxicity profiles

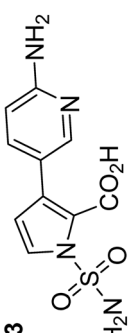
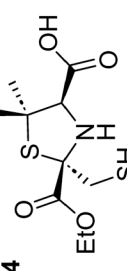
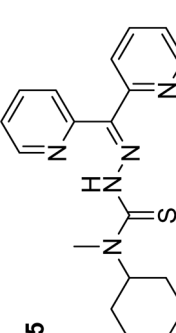
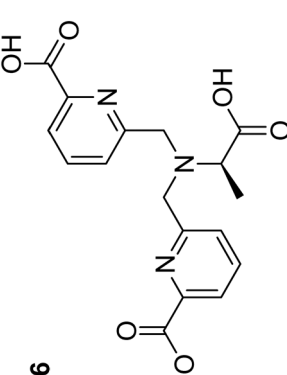
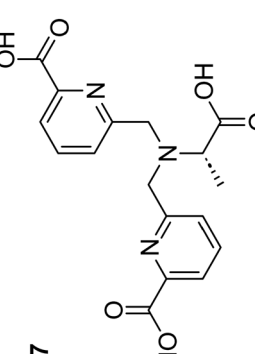
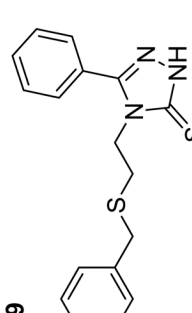
Small molecule	Chemical analogue	Mechanism of action	Activity	Target	Safety profile	Ref.
S3		Pyrrole-carboxylic acid	IC ₅₀ value = 32.37 μM MIC ₅₀ value = 0.06 mg L^{-1}	VIM-2 VIM-1-producing <i>K. pneumoniae</i> clinical isolate	No off-target toxicity CC ₅₀ value > 256 mg L^{-1}	87
S4		Thiazolidine-carboxylic acid	K _i value = 1.9 μM	VIM-2	85% viable HEK293 and L929 cell at >533 μM	88
S5		Thiosemicarbazone	IC ₅₀ value = 0.11 μM IC ₉₀ value = 1 μM MIC _{meropenem+S5} value = 8 $\mu\text{g mL}^{-1}$	VIM-2 VIM-2 VIM-1-producing <i>E. coli</i>	IC ₅₀ value against Vero-E6 between 100 and 200 μM	89
S6		Picolinic acid	IC ₅₀ value = 4.4 μM K _i value = 6.16 μM Meropenem + S6 reduced bacteria population to <10 ² CFU mL^{-1}	VIM-2 VIM-2 VIM-1-producing clinical isolates	HC ₅₀ value > 1024 $\mu\text{g mL}^{-1}$; <i>in vitro</i> showed NOAEL at 32 $\mu\text{g mL}^{-1}$; <i>in vivo</i> showed NOAEL at 50 mg kg^{-1}	90
S7		Picolinic acid	IC ₅₀ value = 4.17 μM K _i value = 5.83 μM Meropenem + S7 reduced bacteria population to <10 ² CFU mL^{-1}	VIM-2 VIM-2 VIM-1-producing clinical isolates	HC ₅₀ value > 1024 $\mu\text{g mL}^{-1}$; <i>in vitro</i> showed NOAEL at 32 $\mu\text{g mL}^{-1}$; <i>in vivo</i> showed NOAEL at 50 mg kg^{-1}	90
S9		Thione	K _i value = 0.21 μM K _i value = 0.24 μM K _i value = 0.21 μM Restored meropenem	VIM-1 VIM-2 VIM-4 VIM-type-producing <i>K. pneumoniae</i> clinical	IC ₅₀ value against HeLa cell = 152 μM ; HC ₅₀ value > 500 μM ; potential off-target toxicity	62

Table 3 (continued)

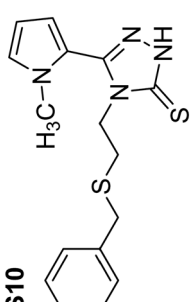
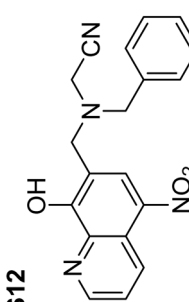
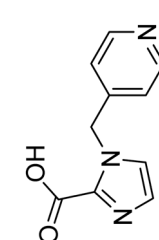
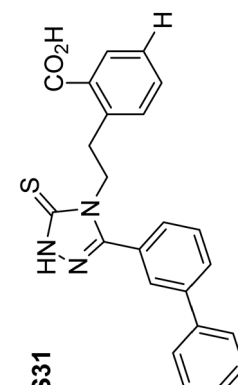
Small molecule	Chemical analogue	Mechanism of action	Activity	Target	Safety profile	Ref.
S10 	Thione	Metal chelator	action K_i value = 0.23 μM K_i value = 0.34 μM K_i value = 0.25 μM Restored meropenem action FIC index value = 0.347	isolates VIM-1 VIM-2 VIM-4 VIM-type-producing <i>K. pneumoniae</i> clinical isolates VIM-type-producing <i>K. pneumoniae</i> clinical isolates	IC ₅₀ value against HeLa cell >250 μM ; HC ₅₀ value > 500 μM	62
S12 	Nitroxoline	Metal chelator	98% enzyme inhibition at 3 μM MIC _{meropenem+S12} value = 0.5 mg L ⁻¹ MIC _{meropenem+S12} value = 1 mg L ⁻¹ MIC _{imipenem+S12} value = 1 mg L ⁻¹ Imipenem + S12 reduced bacteria population >99.9%	VIM-1-producing engineered <i>E. coli</i> strain VIM-1-producing <i>E. coli</i> clinical isolate NDM-5-producing <i>E. coli</i> clinical isolate VIM-1-producing <i>E. coli</i>	IC ₅₀ value against HUVEC cell 45 $\mu\text{g mL}^{-1}$; may induce cell apoptosis	70
S30 	Azole/imidazole-carboxylic acid	Metal chelator	IC ₅₀ value = 0.56 μM IC ₅₀ value = 29.5 μM IC ₅₀ value = 5.78 μM <i>In vitro</i> showed synergism with meropenem FIC index value = 0.05	VIM-2 VIM-1 VIM-5 —	<i>In vivo</i> showed NOAEL at 2000 mg kg ⁻¹	106
S31 	Azole/imidazole-carboxylic acid	Metal chelator	IC ₅₀ value = 1.61 μM IC ₅₀ value = 0.08 μM IC ₅₀ value = 2.1 μM K_i value = 38 nM MIC _{meropenem+S23} value = 2 $\mu\text{g mL}^{-1}$ MIC _{meropenem+S23} value = 1 $\mu\text{g mL}^{-1}$	VIM-type-producing strain VIM-1 VIM-2 VIM-4 VIM-2 VIM-1-producing <i>K. pneumoniae</i> clinical isolates VIM-4-producing <i>K. pneumoniae</i> clinical isolates	Insignificant HeLa cell lysis at 250 μM	19

Table 3 (continued)

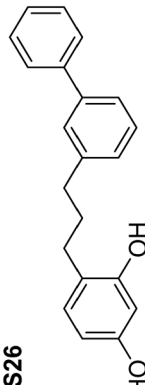
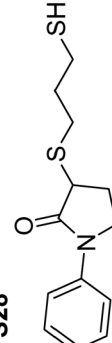
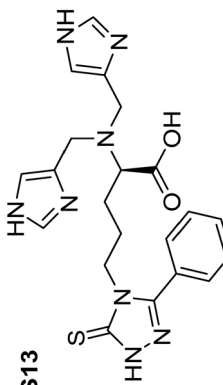
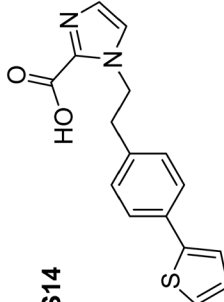
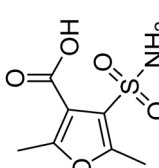
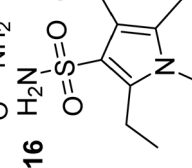
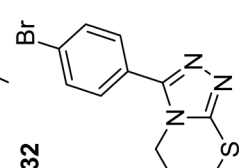
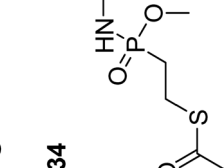
Small molecule	Chemical analogue	Mechanism of action	Activity	Target	Safety profile	Ref.
S26	Phenol 	Metal chelator	mL ⁻¹ IC ₅₀ value = 0.41 μM IC ₅₀ value = 1.3 μM IC ₅₀ value = 0.82 μM MIC _{meropenem} +S25 value = 4 μg mL ⁻¹ MIC _{meropenem} +S25 value = 4 μg mL ⁻¹ IC ₅₀ value = 0.02 μM K _d value < 0.88 μM MIC _{imipenem} +S27 value = 1 μg mL ⁻¹	isolates VIM-1 VIM-2 VIM-4 VIM-1-producing <i>K. pneumoniae</i> clinical isolate VIM-4-producing <i>K. pneumoniae</i> clinical isolate VIM-1 VIM-1	IC ₅₀ value against angiotensin-converting enzyme <100 μM; IC ₅₀ value against HeLa cell >250 μM	102
S28	Pyrrolidinone 	Metal chelator	IC ₅₀ value = 0.02 μM K _d value < 0.88 μM MIC _{imipenem} +S27 value = 1 μg mL ⁻¹	VIM-1 VIM-1 NDM-1-producing engineered <i>E. coli</i>	N/A	104
S13	Thiazolidine-carboxylic acid 	Metal chelator; competitive inhibition	K _i value = 0.15 μM K _i value = 0.41 μM K _i value = 0.20 μM Restored meropenem action MIC _{meropenem} +S28 value = 0.03 μg mL ⁻¹ MIC _{meropenem} +S28 value = 8 μg mL ⁻¹ MIC _{meropenem} +S28 value = 0.03 μg mL ⁻¹ FIC index value = 0.34 IC ₅₀ value = 3.98 μM IC ₅₀ value = 0.018 μM IC ₅₀ value = 0.018 μM MIC _{meropenem} +S29 value ≤ 0.25 μg mL ⁻¹	VIM-1 VIM-2 VIM-4 Multiple VIM-type-producing clinical isolates VIM-1-producing <i>K. pneumoniae</i> clinical isolate VIM-2-producing <i>P. aeruginosa</i> clinical isolate VIM-4-producing <i>K. pneumoniae</i> clinical isolate VIM-type-producing strain VIM-1 VIM-2 VIM-5 VIM-2-producing <i>P. aeruginosa</i> clinical isolate	Metabolically stable IC ₅₀ value against HeLa cell >250 μM No off-target toxicity	92
S14	Azole/imidazole-carboxylic acid 	Metal chelator	IC ₅₀ value = 3.98 μM IC ₅₀ value = 0.018 μM IC ₅₀ value = 0.018 μM MIC _{meropenem} +S29 value ≤ 0.25 μg mL ⁻¹	VIM-1 VIM-2 VIM-5 VIM-2-producing <i>P. aeruginosa</i> clinical isolate	N/A	93

Table 3 (continued)

Small molecule	Chemical analogue	Mechanism of action	Activity	Target	Safety profile	Ref.
S15		Sulfamoylfuran Metal chelator; competitive inhibition	K_i value = 2.81 μM FIC index value = 0.31 FIC index value = 0.19	VIM-2 VIM-2-producing <i>E. coli</i> NDM-1/VIM-1-producing <i>K. pneumonia</i>	IC ₅₀ value against angiotensin-converting enzyme > 1000 μM ; >50% viable HeLa cell at >1000 μM ; LD ₅₀ value on mice model intravenously = 246 mg kg ⁻¹ ; LD ₅₀ value on mice model intraperitoneally >1000 mg kg ⁻¹	94
S16		Sulfamoylfuran Metal chelator; competitive inhibition	K_i value = 0.02 μM MIC _{cefazidim} +S32 value = 1 $\mu\text{g mL}^{-1}$ FIC index value = 0.05	VIM-2 VIM-2-producing engineered <i>E. coli</i> NDM-1/VIM-1-producing <i>K. pneumonia</i>	>50% viable HeLa cell at >1000 μM	94
S32		Thiazine Metal chelator	IC ₅₀ value = 38.36 μM 23% enzyme inhibition at 100 μM 33% enzyme inhibition at 100 μM	VIM-2 VIM-1 VIM-5	N/A	107
S34		Phosphonamidate Metal chelator	IC ₅₀ value = 2.2 μM	VIM-2	N/A	108

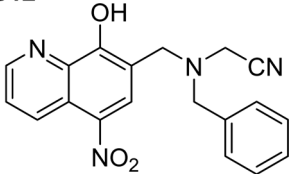
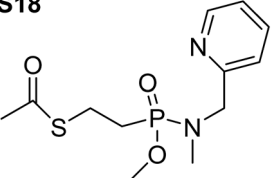
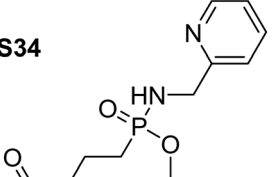
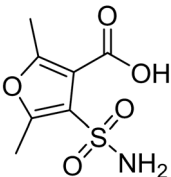
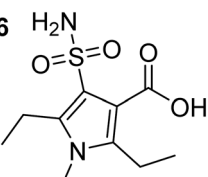
Hint: NOAEL = no-observed-adverse-effect level and NA = not available in report.

investigate its binding mode, molecular docking was used, and S33 appeared to interact with Zn (a metal chelating mechanism) and the VIM-2 residue Asn 210.¹⁰⁸ A summary of the activity data including mechanisms of actions and safety profiles of selected potent small-molecule inhibitors of VIM curated from the recent literature is presented in Table 3.

Inhibitors of other class B carbapenemases and their variants

Impressively, the broad-spectrum inhibitory action of S12 was also evident in the SPM- and GIM-type MBL. The compound S12 (at 32 mg L⁻¹)-imipenem in synergy successfully reduced the MIC values of the clinical isolates of *P. aeruginosa*-SPM-1

Table 4 Summary of selected potent small-molecule inhibitors of other class B carbapenemase, targets, mechanisms of actions and safety profiles

Small molecule	Chemical analogue	Mechanism of action	Activity	Target	Safety profile	Ref.
S12 	Nitroxoline	Metal chelator	MIC _{imipenem+S12} value = 32 mg L ⁻¹ MIC _{imipenem+S12} value = 0.5 mg L ⁻¹	SPM-1-producing <i>P. aeruginosa</i> clinical isolate GIM-1-producing <i>E. cloacae</i> clinical isolate	IC ₅₀ value against HUVEC cell 45 µg mL ⁻¹ May induce cell apoptosis	70
S18 	Phosphoramidate	Binding to loop 5 (93 residues)	IC ₅₀ value = 109 µM	GIM-1	IC ₅₀ value against HeLa cell >500 µM	64
S34 	Phosphoramidate	N/A	IC ₅₀ value = 86 µM	GIM-1	IC ₅₀ value against HeLa cell >500 µM	64
S15 	Sulfamoylfuran	Metal chelator	MIC _{meropenem+S31} value = 0.125 mg L ⁻¹ MIC _{meropenem+S31} value = 0.063 mg L ⁻¹ MIC _{meropenem+S31} value = 2 mg L ⁻¹ MIC _{meropenem+S31} value = 1 mg L ⁻¹ MIC _{meropenem+S31} value = 2 mg L ⁻¹	TMB-2-producing <i>E. coli</i> SPM-1-producing <i>E. coli</i> DIM-1-producing <i>E. coli</i> SIM-1-producing <i>E. coli</i> KHM-1-producing <i>E. coli</i>	IC ₅₀ value against angiotensin-converting enzyme >1000 µM; >50% viable HeLa cell at >1000 µM; LD ₅₀ value on mice intravenously = 246 mg kg ⁻¹ ; LD ₅₀ value on mice model intraperitoneally >1000 mg kg ⁻¹	94
S16 	Sulfamoylfuran	Metal chelator	MIC _{meropenem+S32} value = 0.031 mg L ⁻¹ MIC _{meropenem+S32} value = 0.063 mg L ⁻¹ MIC _{meropenem+S32} value = 0.063 mg L ⁻¹ MIC _{meropenem+S32} value = 0.125 mg L ⁻¹ MIC _{meropenem+S32} value = 0.063 mg L ⁻¹	TMB-2-producing <i>E. coli</i> SPM-1-producing <i>E. coli</i> DIM-1-producing <i>E. coli</i> SIM-1-producing <i>E. coli</i> KHM-1-producing <i>E. coli</i>	>50% viable HeLa cell at >1000 µM	94

Hint: NOAEL = no-observed-adverse-effect level and N/A = not available in report.

and *E. cloacae*-GIM-1 by 16-fold (from 512 to 32 mg L⁻¹) and 4-fold (from 2 to 0.5 mg L⁻¹), respectively.⁷⁰ Palica and colleagues also derivatized a phosphoramidate-based compound, (*R/S*)-(2-(methoxy((pyridin-2-ylmethyl)amino)-

phosphoryl)ethyl) ethanethioate (**S34**), and reported its moderate inhibitory effect on GIM-1 with an IC₅₀ value of 86 μM. The previously mentioned **S18** inhibitor, with an IC₅₀ value of 109 μM was also observed to be active against the GIM-type.

Table 5 Some small-molecule inhibitors of β-lactamases and metallo-β-lactamases currently in various stages of clinical trials

β-Lactamase inhibitor	Other interventions	Primary purpose	Target	Status	Phase	Trial no.
QPX7728 and QPX2014	—	Treatment	BS (KPC, NDM, VIM, <i>etc.</i>)	Completed	1	NCT05072444
QPX7728 and QPX2014	Placebo; individual drug and combination	Treatment	BS (KPC, NDM, VIM, <i>etc.</i>)	Completed	1	NCT04380207
QPX2015	Placebo; oral capsule	Treatment	SBLs, <i>e.g.</i> , KPC	Completed	1	NCT03939429
RPX2014 and RPX7009	Placebo; individual drug and combination	Treatment	SBLs, <i>e.g.</i> , KPC	Completed	1	NCT01897779
RPX7009	Placebo	Treatment	SBLs, <i>e.g.</i> , KPC	Completed	1	NCT01751269
RPX2003	RPX7009	Treatment	SBLs, <i>e.g.</i> , KPC	Completed	1	NCT01772836
RPX2003	Placebo	Treatment	SBLs, <i>e.g.</i> , KPC	Completed	1	NCT01702649
QPX7831 SAD and MAD	Placebo comparator	Treatment	Unspecified BLs	Completed	1	NCT04578873
Vabomere	—	Treatment	SBLs, <i>e.g.</i> , KPC	Recruiting	1	NCT02687906
Vabomere	Best available therapy (BAT)	Treatment	SBLs, <i>e.g.</i> , KPC	Completed	3	NCT02168946
Nacubactam	Meropenem	Treatment	Unspecified BLs	Completed	1	NCT03182504
Imipenem/relebactam (IMI-REL)	—	Susceptibility	BS (KPC, NDM, VIM, IMP, SPM, GIM, <i>etc.</i>)	Enrolling by invitation	—	NCT05285046
IMI-REL	—	Treatment	Unspecified BLs	Completed	1	NCT03230916
Imipenem-cilastatin/relebactam	Active control	Treatment	Unspecified BLs	Recruiting	2, 3	NCT03969901
Imipenem-cilastatin/relebactam	Colistimethate sodium (CMS)	Treatment	Unspecified BLs	Completed	3	NCT02452047
Imipenem-cilastatin/relebactam	—	Treatment	KPC	Withdrawn	4	NCT04785924
Imipenem-cilastatin/relebactam	—	Safety	BS BLs	Recruiting	4	NCT05561764
Piperacillin/tazobactam (Tazocin)	Meropenem	Treatment	ESBLs	Recruiting	4	NCT05355350
Tazocin	Meropenem	Treatment	ESBLs	Recruiting	4	NCT03671967
Tazocin	Other lactams	Treatment	Unspecified BLs	Completed	—	NCT00167960
Tazocin	—	Pharmacokinetics	Unspecified BLs	Completed	—	NCT03738683
Tazocin	—	Health services research	ESBLs	Completed	4	
Tazocin	—	Prevention	ESBLs	Completed	4	NCT00478855
Tazocin (Zosyn)	—	Treatment	Unspecified BLs	Completed	4	NCT00044928
VNRX-5133	VNRX-5022; metronidazole; placebo	Basic science	Unspecified BLs	Completed	1	NCT03332732
Ertapenem	Placebo; zidebactam (WCK 6777)	Treatment	Unspecified BLs	Not yet recruiting	1	NCT05645757
Meropenem	With/without BAT	Treatment	Carbapenemases	Terminated	2, 3	NCT04876430
LP-001	Amoxicillin; clavulanic acid	Treatment	Unspecified BLs	Not yet recruiting	1	NCT05584683
Ceftazidime/avibactam	—	Pharmacokinetics	Unspecified BLs	Completed	—	NCT04358991
Ceftazidime/avibactam	Single dose; multiple dose; cohorts 1–3	Basic science	Unspecified BLs	Terminate	2	NCT04126031
Ceftazidime/avibactam	Colistin	Treatment	Unspecified BLs	Recruiting	3	NCT05258851
Ceftolozane/tazobactam	Various doses and regimens	Treatment	Unspecified BLs	Completed	1	NCT02266706
Ceftriaxone sodium	Sulbactam sodium	Treatment	Unspecified BLs	Completed	4	NCT04202068
Ceftriaxone sodium	Sulbactam sodium	Treatment	Unspecified BLs	Completed	4	NCT04066621
Azactam/Aztreonam	Ceftazidime/avibactam	Treatment	Unspecified BLs	Completed	1, 2	NCT03978091
Aztreonam/avibactam (ATM-AVI)	BAT	Treatment	Unspecified BLs	Not yet recruiting	2	NCT05639647
ATM-AVI	BAT	Treatment	Unspecified BLs	Active, not recruiting	3	NCT03580044
ATM-AVI	Placebo; individual and drug combination	Basic science	Unspecified BLs	Completed	1	NCT01689207
Cefepime	Tazocin; meropenem; vancomycin	Treatment	Unspecified BLs	Not yet recruiting	1	NCT04816968
Augmentin ES-600	—	Treatment	Unspecified BLs	Not yet recruiting	2	NCT05340257

BS: broad-spectrum; BLs: β-lactamases; SBLs: serine β-lactamases; ESBLs: extended spectrum β-lactamases; and data curated from 108 clinical trials.

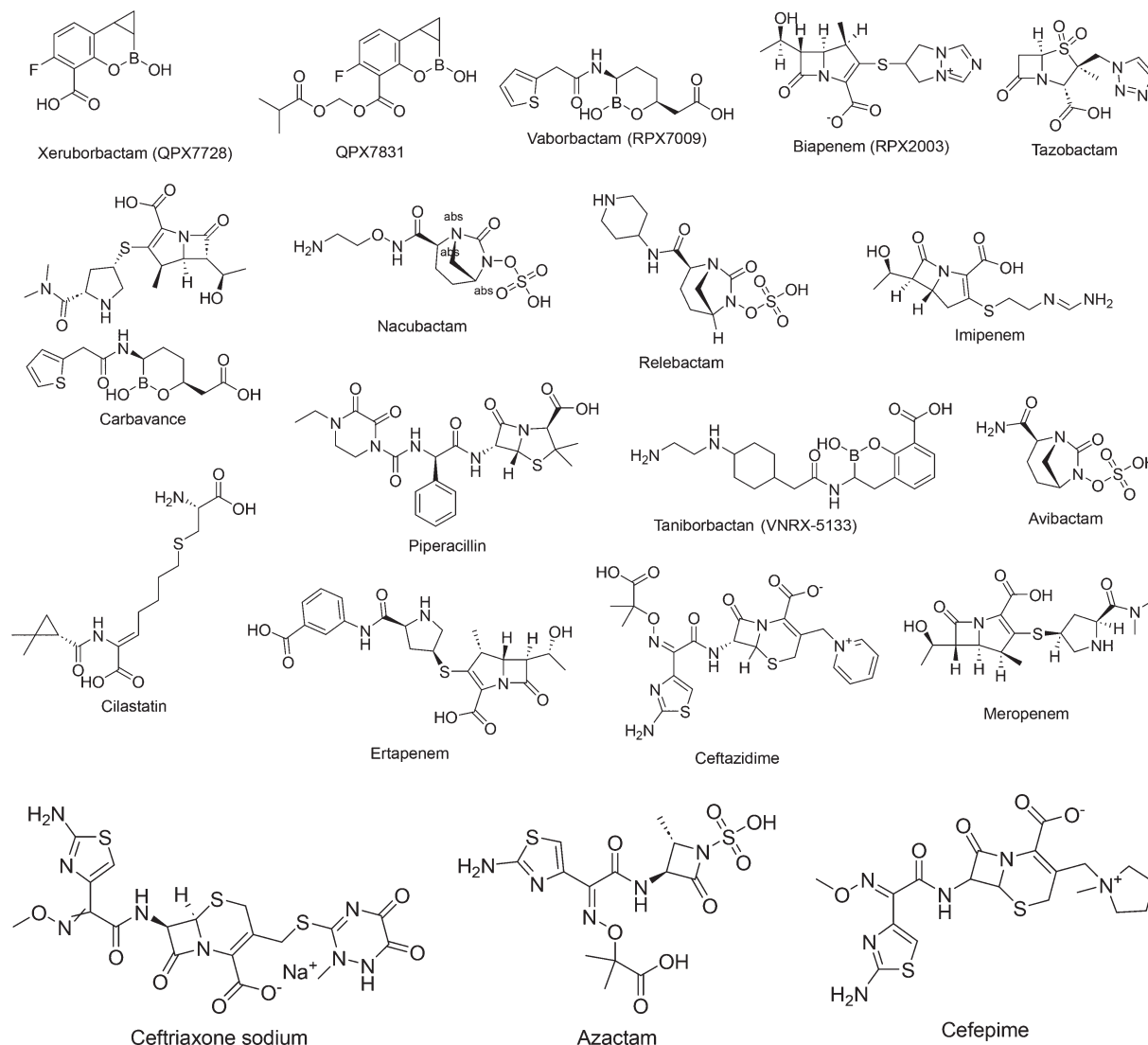


Fig. 3 Chemical structures of some MBL inhibitors in clinical trials.

Similarly to **S18**, **S34** had no meropenem synergy effect but had low cytotoxicity on the HeLa cell line, with an IC_{50} value greater than $500\ \mu\text{M}$.⁶⁴

The broad spectrum inhibitory action of **S15** and **S16** includes the Tripoli MBL (TMB) type, SPM type, Dutch imipenemase (DIM) type, SIM type, and Kyorin hospital MBL (KHM) type. When **S15** (at a concentration of $32\ \mu\text{g mL}^{-1}$) was co-treated with meropenem, the MIC values of meropenem against the *E. coli* strains carrying the respective MBLs, including TMB-2 (from 16 to $0.125\ \mu\text{g mL}^{-1}$), SPM-1 (from 8 to $0.063\ \mu\text{g mL}^{-1}$), DIM-1 (from 8 to $2\ \mu\text{g mL}^{-1}$), SIM-1 (from 16 to $1\ \mu\text{g mL}^{-1}$), and KHM-1 (from 8 to $2\ \mu\text{g mL}^{-1}$), decreased significantly. All these values are decrease between 4- and 128-fold. The MIC values of meropenem against *E. coli* strains carrying the respective MBLs, including TMB-2 (from 16 to $0.031\ \mu\text{g mL}^{-1}$), SPM-1 (from 8 to $0.063\ \mu\text{g mL}^{-1}$), DIM-1 (from 8 to $0.063\ \mu\text{g mL}^{-1}$), SIM-1 (from 16 to $0.125\ \mu\text{g mL}^{-1}$), and KHM-1 (from 8 to $0.063\ \mu\text{g mL}^{-1}$), decreased dramatically in the

presence of **S16** at $32\ \mu\text{g mL}^{-1}$. The MIC values were reduced by up to 516-fold.⁹⁴ A summary of the activity data including the mechanisms of action and safety profiles of promising inhibitors of some other class B carbapenemases from the recent literature is presented in Table 4.

β -Lactamase/metallo- β -lactamase inhibitors in clinical trials

Progressively, some inhibitors of β -lactamases with interesting broad-spectrum potential for treating bacterial infections including critical conditions are currently in various stages of clinical trials for the assessment of their pharmacodynamics, pharmacokinetics, safety and tolerability.¹⁰⁹ They include the cyclic boronates,^{10,110} RPX2014 and RPX7009, QPX7831 SAD and MAD, biapenem, Vabomere, imipenem, meropenem, relebactam/sulbactam, avibactam, aztreonam, piperacillin, tazobactam and several

others in single and combined regimens (Table 5).¹⁰⁹ The chemical structures of the identified MBL inhibitors in clinical trials where applicable are provided in Fig. 3. Detailed information on the inhibitors in clinical trials is available in the NIH US National Library of Medicine (<https://clinicaltrials.gov/ct2/home>) with appropriate links, as presented in Table 5 for further studies.

Conclusion and perspectives

The global threats posed by pathogenic microorganisms on human healthcare and wellness systems, especially related to *Enterobacteriaceae* remain a major concern, thus demanding serious attention, as recognised by WHO. Progressively, scientific efforts have been intensified in recent times towards a better understanding of the mechanisms of AR development by MDR bacteria for strategizing effective therapeutic approaches. Thus far, the restriction of antibiotic intake through the induction of membrane impermeability, overexpression of the drug efflux pumps, biofilm production, suppressive modification of drug targets and production of the β -lactamases have been reported as prominent mechanisms with which the notorious superbugs evade antibiotic actions. Relevantly, the production of MBLs including the IMP, NDM, VIM, GIM, SPM, OXA-48 and KPC by *Enterobacteriaceae* has been recognized as the most minacious mechanism by which almost all classes of antibiotics including the most active β -lactam types are rendered inactivated. The enzymes utilize their metal cofactors and relevant active site residues to hydrolytically coordinate the β -lactam rings of the drugs and induce their opening. Thus, the drugs become inactive, creating critical challenges in clinical settings for managing the infections caused by their producers. Having a comprehensive revelation of the pathways of drug deactivation by the enzyme has significantly supported the development of effective strategies for defeating them. Interestingly, small-molecule ligands with pharmacophoric arrowheads consisting of metal-chelating moieties such as thiol, carbonyl, carboxylate, hydroxyl, amide, amine, and quinine have been identified as promising inhibitors of MBLs. Several analogues containing these active groups have been reported with efficacious suppressive and inhibitory effects on the deadly enzymes, mostly through metal-chelating mechanisms.

Herein, among the hundreds of small-molecule inhibitors of MBL reported from experimental studies within the last three years, 39 analogues consisting of N1–N5 and S1–S34 from natural and synthetic sources, respectively, were overviewed. The natural phytochemicals N1 and N2 potently inhibited NDM individually and in combination and restored the efficacies of carbapenem antibiotic effects on MBL-producing organisms within micromolar concentrations. Notably, N1 and N2 displayed insignificant haemolytic and adverse effects, respectively, indicating their ideal safety in addition to strong inhibitory activities. Interestingly, these compounds can be obtained from vast natural products such

as *A. nidulans*, *A. arborescens*, *W. somnifera*, *M. indica*, *S. officinalis* and common fruits and vegetables for further translational development.

The synthetic analogues mostly contain active electron-rich pharmacophoric scaffolds such as acetamide, azetidinimine, cephalosporin, nicotinamide, nitroxoline, phosphonamide, pyrrolidin, sulfamide and thiosemicarbazone, all supporting metal chelation and efficient MBL deactivation. Among them, the derivatives of sulfamoyl heteroaryl carboxylic acid, S15 and S16, selectively demonstrated broad-spectrum inhibition across a range of MBLs including IMP, and NDM, VIM, SPM, DIM, SIM, TMB and KHM with IC_{50} and K_i values at the micromolar concentration level. These two compounds also attenuated the antibiotic efficacies of meropenem in combination, leading to a reduction in the MIC of the antibiotic by hundreds of folds. Impressively, both compounds showed individual viability of >50% on HeLa cells at >1000 μ M, IC_{50} of >1000 μ M on the angiotensin-converting enzyme and LD_{50} in mice between 246–>1000 mg kg^{-1} , indicating their good safety profiles. Similarly, S12 was reported to exhibit an interesting performance as a potential broad-spectrum MBL inhibitor with activities on IMP, NDM, VIM and GIM, while S3–S7, S9 and S10 and S13–S16 effectively inhibited the three most relevant MBLs, IMP, NDM and VIM, all in micromolar concentrations and mostly with ideal safety profiles, respectively. The mechanisms of actions of the inhibitors have been reported mostly as metal chelation through which the catalytic-inclined metal cofactors are sequestered from the active sites of the MBLs, weakening their potential for deactivating antibiotics. Through their mechanisms, the integrities of antibiotics are adjunctively preserved and synergized for enhanced efficacy. The other mechanisms include the competitive, non-competitive, reversible and allosteric binding for MBL deactivation, oxidative effects on the active site Zn ions and overall potent inhibition of the growth of the MBL-producing pathogens. Progressively, some of the investigated inhibitors mostly among the repurposed current medications and other best available therapy (BAT) have progressed to various stages of clinical trials, advancing the therapeutic breakthrough against AR.

However, most of the experimental studies have been limited to cell-based *in vitro* analyses, through which the physiological responses of the enzyme-bearing organisms to the pharmacological and adverse effects may not be observed. Therefore, the activities of the highlighted compounds deserve to be further investigated *in vivo* to complement their effects on the organisms. Again, the metal-chelating effects of the inhibitors can indiscriminately affect the beneficial human metalloenzymes if not appropriately directed and controlled. Therefore, the acute delivery of metal-chelating small-molecule inhibitors to the targets remains a crucial challenge in furthering the transition of the candidates into real-time medications. Further, the design of the inhibitors into nanostructures can also enhance their acute delivery to

targets through efficient cell permeation in an appropriate dose. Most of the studies have thus far been limited to the basic level of drug design. Thus, concerted scientific efforts are required for their rapid translation into applicable therapeutics to meet the urgent demand for curbing the global menace of AR inclusively through the assessments of pharmacokinetics and pharmacodynamics, and overall drug characteristics of the promising candidates. Some of the inhibitors were also reported to exhibit ability to form ternary complexes upon chelation to the active site metal cofactors. This potentiates the systemic side effects of metal complexes and unpleasant accumulation of metals, which can induce some critical phenomena including carcinogenicity. Thus, effective removal of the resultant complexes remains a challenge for future studies if the interesting inhibitors are to be developed fully into real-life applications.

Nonetheless, in this review, we provided a comprehensive survey of the mechanisms of AR development, especially, regarding the MBLs produced by *Enterobacteriaceae* and appropriate strategies for overcoming the challenges through small-molecule inhibitors. Also, we discussed the effective methods for diagnosing the notorious enzymes and their producers within/outside biological systems. Thus, this represents an ideal template for further studies on the detection and treatment of MDR-related bacterial infections.

CRedit author contributions

The manuscript was written with the contributions of all authors. All authors have approved the final version of the manuscript. Conceptualization, data curation, resources, analysis and writing-original draft, writing-review and editing by YOA; data curation, analysis and writing-original draft by CFC; resources and supervision by MNM.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

This work was financially supported by the Tertiary Education Fund Nigeria, Fundamental Research Grant Scheme, Ministry of Higher Education of Malaysia (grant no. 203.CDADAH.6711955). YOA is thankful to the Universiti Sains Malaysia for Research Fellowship Scheme.

References

- 1 D. Nurjadi, K. Kocer, Q. Chanthalangsy, S. Klein, K. Heeg and S. Boutin, *Antimicrob. Agents Chemother.*, 2022, **66**, e0201121.
- 2 Y. He, S. Zhou, W. Sun, Q. Li, J. Wang and J. Zhang, *J. Global Antimicrob. Resist.*, 2022, **28**, 216–222.
- 3 I. Kyriakidis, E. Vasileiou, Z. D. Pana and A. Tragiannidis, *Pathogens*, 2021, **10**, 1–31.
- 4 Z. Iqbal, J. Sun, H. Yang, J. Ji, L. He, L. Zhai, J. Ji, P. Zhou, D. Tang, Y. Mu, L. Wang and Z. Yang, *Molecules*, 2022, **27**, 3832.
- 5 N. Suthar, V. Pareek, N. Nebhinani and D. Suman, *Indian J. Psychiatry*, 2018, **60**, 145.
- 6 J. A. Karlowsky, S. K. Bouchillon, A. Benaouda, N. Soraa, K. Zerouali, N. Mohamed, T. Alami and D. F. Sahm, *J. Global Antimicrob. Resist.*, 2022, **30**, 23–30.
- 7 CDC, *US Dep. Heal. Hum. Serv.*, 2019, pp. 1–113.
- 8 C. J. Murray, K. S. Ikuta, F. Sharara, L. Swetschinski, G. R. Aguilar, A. Gray, C. Han, C. Bisignano, P. Rao, E. Wool, S. C. Johnson, A. J. Browne, M. G. Chipeta, F. Fell, S. Hackett, G. Haines-Woodhouse, B. H. K. Hamadani, E. A. P. Kumaran, B. McManigal, R. Agarwal, S. Akech, S. Albertson, J. Amuasi, J. Andrews, A. Aravkin, E. Ashley, F. Bailey, S. Baker, B. Basnyat, A. Bekker, R. Bender, A. Bethou, J. Bielicki, S. Boonkasidecha, J. Bukosia, C. Carvalho, C. Castañeda-Orjuela, V. Chansamouth, S. Chaurasia, S. Chiurchiù, F. Chowdhury, A. J. Cook, B. Cooper, T. R. Cressey, E. Criollo-Mora, M. Cunningham, S. Darboe, N. P. J. Day, M. De Luca, K. Dokova, A. Dramowski, S. J. Dunachie, T. Eckmanns, D. Eibach, A. Emami, N. Feasey, N. Fisher-Pearson, K. Forrest, D. Garrett, P. Gastmeier, A. Z. Giref, R. C. Greer, V. Gupta, S. Haller, A. Haselbeck, S. I. Hay, M. Holm, S. Hopkins, K. C. Iregbu, J. Jacobs, D. Jarovsky, F. Javanmardi, M. Khorana, N. Kissoon, E. Kobeissi, T. Kostyaney, F. Krapp, R. Krumkamp, A. Kumar, H. H. Kyu, C. Lim, D. Limmathurotsakul, M. J. Loftus, M. Lunn, J. Ma, N. Mturi, T. Munera-Huertas, P. Musicha, M. M. Mussi-Pinhata, T. Nakamura, R. Nanavati, S. Nangia, P. Newton, C. Ngoun, A. Novotney, D. Nwakanma, C. W. Obiero, A. Olivas-Martinez, P. Olliaro, E. Ooko, E. Ortiz-Brizuela, A. Y. Peleg, C. Perrone, N. Plakkal, A. Ponce-de-Leon, M. Raad, T. Ramdin, A. Riddell, T. Roberts, J. V. Robotham, A. Roca, K. E. Rudd, N. Russell, J. Schnall, J. A. G. Scott, M. Shivamallappa, J. Sifuentes-Osornio, N. Steenkeste, A. J. Stewardson, T. Stoeva, N. Tasak, A. Thaiprakong, G. Thwaites, C. Turner, P. Turner, H. R. van Doorn, S. Velaphi, A. Vongpradith, H. Vu, T. Walsh, S. Waner, T. Wangrangsimaikul, T. Wozniak, P. Zheng, B. Sartorius, A. D. Lopez, A. Stergachis, C. Moore, C. Dolecek and M. Naghavi, *Lancet*, 2022, **399**, 629–655.
- 9 N. Abdul Rahim, Y. Zhu, S. E. Cheah, M. D. Johnson, H. H. Yu, H. E. Sidjabat, M. S. Butler, M. A. Cooper, J. Fu, D. L. Paterson, R. L. Nation, J. D. Boyce, D. J. Creek, P. J. Bergen, T. Velkov and J. Li, *ACS Infect. Dis.*, 2021, **7**, 1584–1595.
- 10 E. Lence and C. González-Bello, *Adv. Ther.*, 2021, **4**, 1–21.
- 11 X. Li, D. Zhao, W. Li, J. Sun and X. Zhang, *Int. J. Mol. Sci.*, 2021, **33**, 197.
- 12 S. Alfei and A. M. Schito, *Pharmaceuticals*, 2022, **15**, 476.
- 13 G. A. Lirio, *Pertanika J. Soc. Sci. Humanit.*, 2022, **30**, 1657–1688.
- 14 Y. O. Ayipo, W. A. Osunniran, H. F. Babamale, M. O. Ayinde and M. N. Mordi, *Coord. Chem. Rev.*, 2022, **453**, 214317.

- 15 M. Mora-Ochomogo and C. T. Lohans, *RSC Med. Chem.*, 2021, **12**, 1623–1639.
- 16 M. Złoch, P. Pomastowski, M. Peer, K. Spärbier, M. Kostrzewa and B. Buszewski, *PLoS One*, 2021, **16**, 1–19.
- 17 E. Romero, S. Oueslati, M. Bencheikroun, A. C. A. D'Hollander, S. Ventre, K. Vijayakumar, C. Minard, C. Exilie, L. Tlili, P. Retailleau, A. Zavala, E. Elisée, E. Selwa, L. A. Nguyen, A. Pruvost, T. Naas, B. I. Iorga, R. H. Dodd and K. Cariou, *Eur. J. Med. Chem.*, 2021, **219**, 113418.
- 18 A. Lucic, T. R. Malla, K. Calvopiña, C. L. Tooke, J. Brem, M. A. McDonough, J. Spencer and C. J. Schofield, *Antibiotics*, 2022, **11**, 1–10.
- 19 F. Verdirosa, L. Gavara, L. Seville, G. Tassone, G. Corsica, A. Legru, G. Feller, G. Chelini, P. S. Mercuri, S. Tanfoni, F. Sannio, M. Benvenuti, G. Cerboni, F. De Luca, E. Bouajila, Y. Vo Hoang, P. Licznar-Fajardo, M. Galleni, C. Pozzi, S. Mangani, J. D. Docquier and J. F. Hernandez, *ChemMedChem*, 2022, **17**, 1–13.
- 20 Y. O. Ayipo, I. Ahmad, W. Alananzeh and A. Lawal, *J. Biomol. Struct. Dyn.*, 2022, 1–21, DOI: [10.1080/07391102.2022.2153168](https://doi.org/10.1080/07391102.2022.2153168).
- 21 J. Z. Arshada and M. Hanif, *RSC Med. Chem.*, 2022, **13**, 1127–1149.
- 22 R. Canton, Y. Doi and P. J. Simner, *Expert Rev. Anti-infect. Ther.*, 2022, **20**, 1077–1094.
- 23 S. M. Diene, L. Pinault, S. A. Baron, S. Azza, N. Armstrong, L. Hadjadj, E. Chabrière, J. M. Rolain, P. Pontarotti and D. Raoult, *Sci. Rep.*, 2021, **11**, 1–8.
- 24 A. Ruedas-lópez, I. Alonso-garcía, C. Lasarte-monterrubio, P. Guijarro-sánchez, E. Gato, J. M. Gutiérrez-urbón, M. Oviaño, A. Beceiro, C. González-bello, A. Oliver, J. Arca-Suárez and G. Bou, *Antimicrob. Agents Chemother.*, 2022, **66**, 1–14.
- 25 Y. Zhai, S. Lee, L. Teng, Z. Ma, N. B. Hilliard, R. J. May, S. A. Brown, F. Yu, K. E. Desear, K. Cherabuddi, K. H. Rand, J. G. Morris, N. M. Iovine and K. C. Jeong, *JAC Antimicrob. Resist.*, 2021, **3**, dlab032.
- 26 J. Denissen, B. Reyneke, M. Waso-Reyneke, B. Havenga, T. Barnard, S. Khan and W. Khan, *Int. J. Hyg. Environ. Health*, 2022, **244**, 114006.
- 27 M. Din, K. M. Babar, D. Shah, G. Sarwar, S. Khan, H. Sadaf, S. S. Ahmed, F. L. Ali, M. Yasir and N. Ahmed, *J. Pak. Med. Assoc.*, 2021, **71**, 228–232.
- 28 S. Naeem, H. Bilal, H. Muhammad, M. A. Khan, F. Hameed, S. Bahadur and T. U. Rehman, *J. Infect. Dev. Countries*, 2021, **15**, 516–522.
- 29 S. Maraki, V. E. Mavromanolaki, P. Moraitis, D. Stafylaki and A. Kasimati, *Eur. J. Clin. Microbiol. Infect. Dis.*, 2021, **40**, 1755–1759.
- 30 B. D. Johnston, P. Thuras, S. B. Porter, M. Castanheira and J. R. Johnson, *J. Global Antimicrob. Resist.*, 2021, **24**, 190–197.
- 31 R. Chowdary and S. Sushma, *Food Res.*, 2021, **5**, 144–151.
- 32 Z. Messasma, D. Aggoun, S. Houchi, A. Ourari, Y. Ouennoughi, F. Keffous and R. Mahdadi, *J. Mol. Struct.*, 2021, **1228**, 129463.
- 33 A. Vahhabi, A. Hasani, M. A. Rezaee, B. Baradaran, A. Hasani, H. Samadi Kafil, F. Abbaszadeh and L. Dehghani, *J. Chemother.*, 2021, **33**, 137–155.
- 34 W. C. Reygaert, *AIMS Microbiol.*, 2018, **4**, 482–501.
- 35 S. Liu, N. Huang, C. Zhou, Y. Lin, Y. Zhang, L. Wang, X. Zheng, T. Zhou and Z. Wang, *Infect. Drug Resist.*, 2021, **14**, 3647–3658.
- 36 Y. Suzuki, T. Sato, Y. Fukushima, C. Nakajima, Y. Suzuki, S. Takahashi and S. I. Yokota, *Int. J. Antimicrob. Agents*, 2020, **55**, 105919.
- 37 Y. H. Ku and W. L. Yu, *Infect., Genet. Evol.*, 2021, **88**, 104707.
- 38 O. Lomovskaya, D. Rubio-Aparicio, R. Tsivkovski, J. Loutit and M. Dudley, *Antimicrob. Agents Chemother.*, 2022, **66**, e0216821.
- 39 X. Pan, X. Zhao, Y. Song, H. Ren, Z. Tian, Q. Liang, Y. Jin, F. Bai, Z. Cheng, J. Feng and W. Wu, *Microbiol. Spectrum*, 2022, **10**(1), e02678-21.
- 40 M. Zalacain, C. Lozano, A. Llanos, N. Sprynski, T. Valmont, C. De Piano, D. Davies, S. Leires, C. Sable, A. Ledoux, I. Morrissey, M. Lemonnier and M. Everett, *Antimicrob. Agents Chemother.*, 2021, **65**, e00203–e00221.
- 41 S. D. Shukla, G. Gupta, R. Khatoon, R. Jain and A. Gupta, *J. Pure Appl. Microbiol.*, 2022, **16**, 858–866.
- 42 M. Ratajczak, D. Kaminska, J. Długaszczyńska and M. Gajecka, *Pathogens*, 2021, **10**, 1–13.
- 43 M. Al-Bayati and S. Samarasinghe, *Int. J. Environ. Res. Public Health*, 2022, **19**, 4788.
- 44 W. Qian, X. Li, M. Yang, C. Liu, Y. Kong, Y. Li, T. Wang and Q. Zhang, *Infect. Drug Resist.*, 2022, **15**, 2865–2878.
- 45 P. Sreenivasan, B. Sharma, S. Kaur, S. Rana, M. Biswal, P. Ray and A. Angrup, *J. Antibiot.*, 2022, 454–462.
- 46 L. Hu, H. Yang, T. Yu, F. Chen, R. Liu, S. Xue, S. Zhang, W. Mao, C. Ji, H. Wang and H. Xie, *Eur. J. Med. Chem.*, 2022, **232**, 114174.
- 47 K. Bush, *Antimicrob. Agents Chemother.*, 2018, **62**, e01076-18.
- 48 K. Bush and G. A. Jacoby, *Antimicrob. Agents Chemother.*, 2010, **54**, 969–976.
- 49 V. G. Grigorenko, M. G. Khrenova, I. P. Andreeva, M. Y. Rubtsova, A. I. Lev, T. S. Novikova, E. V. Detusheva, N. K. Fursova, I. A. Dyatlov and A. M. Egorov, *Int. J. Mol. Sci.*, 2022, **23**, 1834.
- 50 Y. Ge, P. W. Kang, J. Q. Li, H. Gao, L. Zhai, L. Y. Sun, C. Chen and K. W. Yang, *J. Antibiot.*, 2021, **74**, 574–579.
- 51 N. Wade, K. H. M. E. Tehrani, N. C. Bruchle, M. J. van Haren, V. Mashayekhi and N. I. Martin, *ChemMedChem*, 2021, **16**, 1651–1659.
- 52 A. J. M. Farley, Y. Ermolovich, K. Calvopiña, P. Rabe, T. Panduwawala, J. Brem, F. Björklund and C. J. Schofield, *ACS Infect. Dis.*, 2021, **7**, 1809–1817.
- 53 M. Sadek, A. M. Soliman, H. Nariya, T. Shimamoto and T. Shimamoto, *Microb. Drug Resist.*, 2021, **27**, 196–203.
- 54 Y. H. Yan, G. Li and G. B. Li, *Med. Res. Rev.*, 2020, **40**, 1558–1592.

- 55 Y. Yang, Y.-H. Yan, C. J. Schofield, A. McNally, Z. Zong and G.-B. Li, *Trends Microbiol.*, 2023, **xx**, 1–14.
- 56 Z. Cheng, C. R. Bethel, P. W. Thomas, B. A. Shurina, J. P. Alao, C. A. Thomas, K. Yang, S. H. Marshall, H. Zhang, A. M. Sturgill, A. N. Kravats, R. C. Page, W. Fast, R. A. Bonomo and M. W. Crowder, *Antimicrob. Agents Chemother.*, 2021, **65**, e01714.
- 57 T. Naas, S. Oueslati, R. A. Bonnin, M. L. Dabos, A. Zavala, L. Dortet, P. Retailleau and B. I. Iorga, *J. Enzyme Inhib. Med. Chem.*, 2017, **32**, 917–919.
- 58 H. Zhang, K. Yang, Z. Cheng, C. Thomas, A. Steinbrunner, C. Pryor, M. Vulcan, C. Kemp, D. Orea, C. Paththamperuma, A. Y. Chen, S. M. Cohen, R. C. Page, D. L. Tierney and M. W. Crowder, *Bioorg. Med. Chem.*, 2021, **40**, 116183.
- 59 Y. Yamagishi, N. Nakayama, N. Matsunaga, D. Sakanashi, H. Suematsu, Y. Matsumoto and H. Mikamo, *J. Infect. Chemother.*, 2022, **28**, 526–531.
- 60 K. A. Cairns, V. Hall, G. E. Martin, D. W. J. Griffin, J. D. Stewart, S. F. Khan, I. J. Abbott, Z. Meher-Homji, C. O. Morrissey, C. Sia, J. Love, C. E. Corallo, P. Bergin, A. Sharma, G. Basu, A. Spencer and A. Y. Peleg, *Transplant Infect. Dis.*, 2021, **23**, 0–2.
- 61 J. N. O'Donnell, V. Putra, G. M. Belfiore, B. L. Maring, K. Young and T. P. Lodise, *Int. J. Antimicrob. Agents*, 2022, **59**, 106595.
- 62 A. Legru, F. Verdirosa, J. F. Hernandez, G. Tassone, F. Sannio, M. Benvenuti, P. A. Conde, G. Bossis, C. A. Thomas, M. W. Crowder, M. Dillenberger, K. Becker, C. Pozzi, S. Mangani, J. D. Docquier and L. Gavara, *Eur. J. Med. Chem.*, 2021, **226**, 113873.
- 63 S. Price, R. Mehta, D. Tan, A. Hinojosa, P. W. Thomas, T. Cummings, W. Fast and E. L. Que, *J. Inorg. Biochem.*, 2022, **233**, 111869.
- 64 K. Palica, M. Voráčová, S. Skagseth, A. A. Rasmussen, L. Allander, M. Hubert, L. Sandegren, H. K. Schröder Leiros, H. Andersson and M. Erdélyi, *ACS Omega*, 2022, **7**, 4550–4562.
- 65 C. Chen, K. Yang, L. Zhai, H. Ding and J. Chigan, *Bioorg. Chem.*, 2022, **118**, 105474.
- 66 M. F. Mojica, M. A. Rossi, A. J. Vila and R. A. Bonomo, *Lancet Infect. Dis.*, 2022, **22**, e28–e34.
- 67 L. H. E. Wieske, J. Bogaerts, A. A. M. Leding, S. Wilcox, A. Andersson Rasmussen, K. Leszczak, L. Turunen, W. A. Herrebout, M. Hubert, A. Bayer and M. Erdélyi, *ACS Med. Chem. Lett.*, 2022, **13**, 257–261.
- 68 B. Kar, C. N. Kundu, S. Pati and D. Bhattacharya, *J. Biomol. Struct. Dyn.*, 2021, 1–14.
- 69 K. Kwapien, L. Gavara, J. D. Docquier, D. Berthomieu, J. F. Hernandez and N. Gresh, *J. Comput. Chem.*, 2021, **42**, 86–106.
- 70 A. Proschak, G. Martinelli, D. Frank, M. J. Rotter, S. Brunst, L. Weizel, L. D. Burgers, R. Fürst, E. Proschak, I. Sosič, S. Gobec and T. A. Wichelhaus, *Eur. J. Med. Chem.*, 2022, **228**, 113975.
- 71 A. U. Khan, L. Maryam and R. Zarrilli, *BMC Microbiol.*, 2017, **17**, 1–12.
- 72 S. E. Boyd, D. M. Livermore, D. C. Hooper and W. W. Hope, *Antimicrob. Agents Chemother.*, 2020, **64**, 1–20.
- 73 M. Wilamowski, D. A. Sherrell, Y. Kim, A. Lavens, R. W. Henning, K. Lazarski, A. Shigemoto, M. Endres, N. Maltseva, G. Babnigg, S. C. Burdette, V. Srajer and A. Joachimiak, *Nat. Commun.*, 2022, **13**, 7379.
- 74 M. I. Abboud, M. Kosmopoulou, A. P. Krismanich, J. W. Johnson, P. Hinchliffe, J. Brem, T. D. W. Claridge, J. Spencer, C. J. Schofield and G. I. Dmitrienko, *Chem. – Eur. J.*, 2018, **24**, 5734–5737.
- 75 J. H. Toney, G. G. Hammond, P. M. D. Fitzgerald, N. Sharma, J. M. Balkovec, G. P. Rouen, S. H. Olson, M. L. Hammond, M. L. Greenlee and Y. D. Gao, *J. Biol. Chem.*, 2001, **276**, 31913–31918.
- 76 H. Feng, X. Liu, S. Wang, J. Fleming, D. C. Wang and W. Liu, *Nat. Commun.*, 2017, **8**, 2242.
- 77 R. Salimraj, P. Hinchliffe, M. Kosmopoulou, J. M. Tyrrell, J. Brem, S. S. van Berkel, A. Verma, R. J. Owens, M. A. McDonough, T. R. Walsh, C. J. Schofield and J. Spencer, *FEBS J.*, 2019, **286**, 169–183.
- 78 G. Bianco, M. Boattini, M. Iannaccone, E. Zanotto, R. Cavallo and C. Costa, *Microb. Drug Resist.*, 2021, **27**, 740–746.
- 79 S. M. Rudresh, Basavaraj, M. N. Kusuma and G. S. Ravi, *J. Clin. Diagn. Res.*, 2022, **16**, 18–22.
- 80 G. Li, Z. Ye, W. Zhang, N. Chen, Y. Ye, Y. Wang, F. Wu, K. Wang and L. Fan, *Eur. J. Clin. Microbiol. Infect. Dis.*, 2022, **41**, 815–825.
- 81 C. López, J. Delmonti, R. A. Bonomo and A. J. Vila, *J. Biol. Chem.*, 2022, 101665.
- 82 A. C. Jackson, T. B. J. Pinter, D. C. Talley, A. Baker-Agha, D. Patel, P. J. Smith and K. J. Franz, *ChemMedChem*, 2021, **16**, 654–661.
- 83 F. S. Aljohani, N. Rezki, M. R. Aouad, M. Hagar, B. A. Bakr, M. M. Shaaban and B. H. Elwakil, *Antibiotics*, 2022, **11**, 1–22.
- 84 P. B. Chouke, K. M. Dadure, A. K. Potbhare, G. S. Bhusari, A. Mondal, K. Chaudhary, V. Singh, M. F. Desimone, R. G. Chaudhary and D. T. Masram, *ACS Omega*, 2022, **7**, 20983–20993.
- 85 A. Kobayashi, Y. Yamaguchi, S. Maruki, S. Kim, Y. Arakawa and S. Kurihara, *Mater. Lett.*, 2022, **315**, 131901.
- 86 V. K. Mulligan, S. Workman, T. Sun, S. Rettie, X. Li, L. J. Worrall, T. W. Craven, D. T. King, P. Hosseinzadeh, A. M. Watkins, P. Douglas Renfrew, S. Guffy, J. W. Labonte, R. Moretti, R. Bonneau, N. C. J. Strynadka and D. Baker, *Proc. Natl. Acad. Sci. U. S. A.*, 2021, **118**, e2012800118.
- 87 N. Ooi, V. E. Lee, N. Chalam-Judge, R. Newman, A. J. Wilkinson, I. R. Cooper, D. Orr, S. Lee and V. J. Savage, *J. Antimicrob. Chemother.*, 2021, **76**, 460–466.
- 88 M. A. Rossi, V. Martinez, P. Hinchliffe, M. F. Mojica, V. Castillo, D. M. Moreno, R. Smith, B. Spellberg, G. L. Drusano, C. Banchio, R. A. Bonomo, J. Spencer, A. J. Vila and G. Mahler, *Chem. Sci.*, 2021, **12**, 2898–2908.
- 89 J. Q. Li, H. Gao, L. Zhai, L. Y. Sun, C. Chen, J. Z. Chigan, H. H. Ding and K. W. Yang, *Bioorg. Med. Chem.*, 2021, **38**, 116128.

- 90 F. Chen, M. Bai, W. Liu, H. Kong, T. Zhang, H. Yao, E. Zhang, J. Du and S. Qin, *Eur. J. Med. Chem.*, 2021, **224**, 113702.
- 91 K. H. M. E. Tehrani, N. Wade, V. Mashayekhi, N. C. Bröchle, W. Jespers, K. Voskuil, D. Pesce, M. J. Van Haren, G. J. P. Van Westen and N. I. Martin, *J. Med. Chem.*, 2021, **64**, 9141–9151.
- 92 A. Legru, F. Verdirrosa, Y. Vo-Hoang, G. Tassone, F. Vascon, C. A. Thomas, F. Sannio, G. Corsica, M. Benvenuti, G. Feller, R. Coulon, F. Marcoccia, S. R. Devente, E. Bouajila, C. Piveteau, F. Leroux, R. Deprez-Poulain, B. Deprez, P. Licznar-Fajardo, M. C. Crowder, L. Cendron, C. Pozzi, S. Mangani, J.-D. Docquier, J.-F. Hernandez and L. Gavara, *J. Med. Chem.*, 2022, **65**, 16392–16419.
- 93 R. Li, H. Su, W. Chen, Y. H. Yan, C. Zhou, L. Mou, H. Yang, S. Qian, Z. Wang, L. Yang and G. B. Li, *Bioorg. Med. Chem.*, 2022, **72**, 116993.
- 94 J. Wachino, W. Jin, K. Kimura, H. Kurosaki, A. Sato and Y. Arakawa, *MBio*, 2020, **11**, 1–17.
- 95 Y. Guo, Y. Yang, X. Xu, L. Li, Y. Zhou, G. Jia, L. Wei, Q. Yu and J. Wang, *Eur. J. Med. Chem.*, 2022, **231**, 114108.
- 96 A. Vasudevan, D. K. Kesavan, L. Wu, Z. Su, S. Wang, M. K. Ramasamy, W. Hopper and H. Xu, *BioMed Res. Int.*, 2022, **2022**, 4230788.
- 97 Y. Yang, Y. Guo, Y. Zhou, Y. Gao, X. Wang and J. Wang, *Front. Pharmacol.*, 2020, **11**, 1–11.
- 98 J. Q. Li, L. Y. Sun, Z. Jiang, C. Chen, H. Gao, J. Z. Chigan, H. H. Ding and K. W. Yang, *Bioorg. Chem.*, 2021, **107**, 104576.
- 99 J. Chigan, Z. Hu, L. Liu, Y. Xu, H. Ding and K. Yang, *Bioorg. Chem.*, 2022, **120**, 105654.
- 100 W. B. Jin, C. Xu, X. L. Qi, P. Zeng, W. Gao, K. H. Lai, J. Chiou, E. W. C. Chan, Y. C. Leung, T. H. Chan, K. Y. Wong, S. Chen and K. F. Chan, *New J. Chem.*, 2021, **45**, 3515–3534.
- 101 B. Zhao, X. Zhang, T. Yu, Y. Liu, X. Zhang, Y. Yao, X. Feng, H. Liu, D. Yu, L. Ma and S. Qin, *Acta Pharm. Sin. B*, 2021, **11**, 203–221.
- 102 L. Gavara, F. Verdirrosa, L. Seville, A. Legru, G. Corsica, L. Nauton, P. S. Mercuri, F. Sannio, F. De Luca, M. Hadjadj, G. Cerboni, Y. Vo Hoang, P. Licznar-Fajardo, M. Galleni, J. D. Docquier and J. F. Hernandez, *Bioorg. Med. Chem.*, 2022, **72**, 116964.
- 103 A. C. Jackson, J. M. Zaengle-Barone, E. A. Puccio and K. J. Franz, *ACS Infect. Dis.*, 2020, **6**, 1264–1272.
- 104 M. Krasavin, D. Zhukovsky, I. Solovyev, D. Barkhatova, D. Dar'in, D. Frank, G. Martinelli, L. Weizel, A. Proschak, M. Rotter, J. S. Kramer, S. Brunst, T. A. Wichelhaus and E. Proschak, *ChemMedChem*, 2021, **16**, 3410–3417.
- 105 Ø. Samuelsen, O. A. H. Åstrand, C. Fröhlich, A. Heikal, S. Skagseth, T. J. O. Carlsen, H. K. S. Leiros, A. Bayer, C. Schnaars, G. Kildahl-Andersen, S. Lauksund, S. Finke, S. Huber, T. Gjøn, A. M. S. Andresen, O. A. Økstad and P. Rongved, Zn148 is a modular synthetic metallo- β -lactamase inhibitor that reverses carbapenem resistance in Gram-negative pathogens in vivo, *Antimicrob. Agents Chemother.*, 2020, **64**, e02415.
- 106 Y. H. Yan, W. Li, W. Chen, C. Li, K. R. Zhu, J. Deng, Q. Q. Dai, L. L. Yang, Z. Wang and G. B. Li, *Eur. J. Med. Chem.*, 2022, **228**, 113965.
- 107 C. Yuan, J. Yan, C. Song, F. Yang, C. Li, C. Wang, H. Su, W. Chen, L. Wang, Z. Wang, S. Qian and L. Yang, *Molecules*, 2020, **25**, 1–15.
- 108 Y. L. Zhang, Y. Yan, X. J. Wang and K. W. Yang, *Antibiotics*, 2020, **9**, 9–11.
- 109 Clinical Trials, Clinical trials of β -lactamase inhibitors for bacterial infections, <https://clinicaltrials.gov/ct2/results?cond=Bacterial+Infections&term=lactamase+inhibitor&cntry=&state=&city=&dist=>, (accessed 22 January 2023).
- 110 O. Lomovskaya, R. Tsivkovski, D. Sun, R. Reddy, M. Totrov, S. Hecker, D. Griffith, J. Loutit and M. Dudley, *Front. Microbiol.*, 2021, **12**, 697180.