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Structural Insights and role of Metallo-B-Lactamase Inhibitors

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Abstract - The breakdown of the β-lactam ring is catalyzed by metallo-β-lactamases (MBLs), which in turn impart resistance to carbapenems and other β-lactam antibiotics. Due to a lack of adequate treatment options, the fast spread of MBL-producing bacteria poses a serious threat to public health. The creation of effective MBL has therefore been the focus of much study inhibitors capable of restoring β-lactam efficacy. This study provides a comprehensive review of the structural features of MBLs, with a focus regarding the design of the active site and the coordination of metal ions, which are critical for the enzyme's catalytic activity. A common catalytic mechanism with comparable reaction species is shared by MBLs, notwithstanding their structural diversity. In this article, we detail a variety of MBL inhibitors that imitate substrate, transition state, intermediate, and product species that are produced during hydrolysis. Within the context of MBLs' mechanism, new inhibitors based on boron and thiol have recently been developed, and we take a look at their development. We may also bring up Zn(II) ions are necessary for substrate binding and catalysis, making chelators a potential tactic.

Keywords: metallo-β-lactamases; mechanism-based inhibitors; antibiotic resistance; reaction mechanism

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INTRODUCTION

Laxminarayan et al. (2013) and Yan et al. (2020) both agree that antibiotics are vital to contemporary healthcare systems. Antibiotics are often prescribed before surgical procedures in hospitals to ensure the safety of patients. The widespread use of antibiotics in animal husbandry, agriculture, and food production, as well as the subsequent contamination of the environment with these drugs, puts pressure on bacterial communities to evolve resistance to antibiotics and leads to the spread of MDR bacteria (Hou et al., 2017). There has been a dramatic decline in the number of new medications with unique action mechanisms throughout the previous few decades, coinciding with the worrisome incidence of multidrug-resistant bacterial growth. The rising incidence of microorganisms resistant to antibiotics and the exorbitant expense of research and development, major pharmaceutical companies have shifted their focus away from antibiotic medication development and onto more lucrative areas, such cancer therapies. According to de Kraker et al. (2016), if the present trend continues over the next half-century, multidrug-resistant bacterial illnesses would kill more people than cancer.

Because bacteria do not have or use structures or routes that are similar to those in humans, antibacterials work by blocking these structures and processes. New multidrug-resistant (MDR) bacterial strains emerge, however, from the vast bacterial population and the ease of producing new mutant strains owing to fast reproduction following repeated and persistent antibiotic treatment (Berendonk et al., 2015; Blair et al., 2015). Bacterial drug resistance is challenging to combat because mutations and resistance genes may spread quickly via DNA. These procedures diminish the value of an effective new antibiotic medicine and discourage investment in similar medicines.

Since there isn't a clinically approved MBL inhibitor at the moment, further research and innovation are required in this area. The best way to utilize an MBL inhibitor β-lactam antibiotics are manufactured as a

component of combination treatment work again against bacteria that have an MBL gene but are resistant to them. Despite the lack of a human-approved MBL inhibitor, several inhibitors with different structural and mechanistic properties have been published in recent years in scholarly journals and patent filings. The structures of these MBL inhibitors that have been characterized include a wide variety of pharmacophores. Deactivation of MBLs may occur when these inhibitors connect with the MBL active site and bind to zinc, or when they sequester zinc. It is necessary to use strong metal chelators that aggressively remove zinc ions from the MBL active site in order to effectively inhibit the enzyme's ability to hydrolyze the βlactam ring. Another alternative is to use a small molecule MBL inhibitor; these inhibitors do not really remove zinc from the enzyme, but they do coordinate the metal ion inside the active site, which blocks catalysis by separating the activated water molecule.

There are a lot of moving parts in the issue of developing inhibitors that work for all MBL subtypes. Since MBLs are metallo-enzymes, It seems to reason that the optimal strategy would include identifying inhibitors capable of binding or chelating the active site's zinc ions. Incorporating zinc binding medicines in vivo is very risky due to the possibility of off-target effects caused by the several metallo-enzymes involved in human metabolism. I am familiar with the material Thiol and thiocarbonyl compounds were among the first to be identified as MBL inhibitors; these compounds showed strong enzyme inhibition for many MBLs in vitro. Thiols have the potential to be effective in therapeutic settings, however their quick oxidation to homo- and hetero-disulfides in biological systems may compromise this. An ideal MBL inhibitor would not only be stable enough to be used in vivo, but it would also not interfere with other important metallo-enzymes' ability to bind to metals that are necessary for their function.

LITERATURE REVIEW

Timothy Palzkill (2023), An increasingly pressing problem is the development of resistance to the antibacterial agents most often used, antibiotics that are β-lactam. A group of enzymes known as metho-β-lactamases are responsible for hydrolyzing several β-lactam medicines, including carbapenems. The fact that the enzyme processes vary based on the β-lactamase subclass and whether one or two zincs are bound in the active site reflects this variety. Because of their extensive distribution, these genes that encode enzymes have become a key component of Gram-negative bacteria's resistance mechanisms. Moreover, there are currently no inhibitors intended for therapeutic use that can halt the action of metallo-β-lactamase. This study compiles the key points from the many investigations that have illuminated these enzymes' structure, function, and action mechanism.

As per the research conducted by in 2023, the researchers Keizo Yamamoto, Hideaki Tanaka, Genji Kurisu, Ryuichi Nakano, Hisakazu Yano, and Hiromi Sakai hypothesized that carbapenem and other β-lactam antibiotic resistance may be caused by the presence of IMP-type metallo-β-lactamases. With the exception of a single point mutation, IMP-1 and IMP-6 are identical; IMP-6 contains Gly262 while IMP-1 has Ser262. Imipenem and meropenem have very comparable kcat/Km values for IMP-1; however, meropenem has a kcat/Km that is seven times greater than imipenem's for IMP-6. In actual clinical settings, this might lead to a treatment plan that isn't successful and, ultimately, unsuccessful therapeutic outcome. Here we show the crystal structures of IMP-6 and IMP-1, two molecules in the same space group with comparable cell constants of 1.70 and 1.94 Å, respectively. From an overarching design perspective, IMP-1 and IMP-6 are quite similar. In IMP-6, the substrate-binding loop region—residues 60–66—is more malleable compared to IMP-1. Imipenem and meropenem substrate specificity are determined by this difference in flexibility of IMP-type metallo-P-lactamases. The hydrophobic interaction between Pro68 and the amino acid at position 262 changes the mobility of His263 in IMP-type metallo-β-lactamases, which in turn changes the flexibility of the loop. The Km of IMP-6 for imipenem was increased when Pro68 was substituted with a glycine, while the affinity for meropenem was unaffected.

In a 2018 publication by Docquier and Mangani, The rise of bacteria that are resistant to antibiotics and other drugs presents a serious threat a serious danger to our ability to treat bacterial infections, especially those that may be fatal, such as those that are acquired in healthcare facilities. New antibacterial medications, especially those that target Gram-negative bacteria, are urgently needed in the medical field, and this growing issue requires several suitable solutions. Given the clinical relevance of β-lactam antibiotics and β-lactamase-mediated 30 resistance, and considering the vast structural and mechanistic variety among prospective β-lactamase targets, it seems particularly attractive to identify and create combinations that include a β-lactamase inhibitor. This study will go over how β-lactamase inhibitors that are now on the market have changed over time,

as well as the latest research that has produced novel β-lactamase inhibitors that might be useful in clinical settings or are currently in the 35th stage of clinical development.

Gangadharappa, B. S., Sharath, R., Revanasiddappa, P. D., Chandramohan, V., Balasubramaniam, M., & Vardhineni, T. P. (2019), Enzymes belonging to the class known as metho-beta-lactamase (MBL) facilitate the hydrolysis of certain beta-lactam antibiotics, which in turn causes bacteria to become resistant to these drugs. Therefore, efforts are being made to inhibit MBL as a possible strategy to render bacteria more susceptible using antibiotics that are beta-lactam type. Natural MBL inhibitors including eupalitin, rosmarinic acid, and luteolin are instead the focus of this research. The apo-protein, which was found in the crystal structure of MBL as hydrolyzed Meropenem, was formed by undocking from the active core pocket. To provide the groundwork for the present experiment and draw conclusions, the apo-protein was re-docked with substrate, three recognized MBL inhibitors, and natural chemicals. The efficacy of naturally occurring inhibitors was further investigated by analyzing the enzyme's dynamic behavior across simulated time using molecular dynamics investigations. According to our findings, when a natural inhibitor was present, the MBL enzyme took on a different structural state. This is because the naturally occurring inhibitors attempted to occupy a different binding location on the enzyme, therefore disrupting its active core pocket. The binding pocket includes the active site pocket as well as a recently identified ligand area, the substrate should not occupy the active site. Therefore, things that inhibit MBL naturally might be a promising target.

In 2016, Brem et al. discovered that bacteria because of β-lactamases, they can withstand almost all β-lactam antibiotics. In significant research, it was shown that acyclic boronic acids might operate as "transition state analogues" to inhibit nucleophilic serine enzymes like serine-β-lactamases. The results of our biochemical and biophysical investigations show that cyclic boronates effectively block β-lactamases that are reliant on zinc or nucleophilic serine via a process that involves imitating the common tetrahedral intermediate. Following the same mode of action, cyclic boronates also have a strong inhibitory effect on the PBP5, a penicillin-binding protein that is not necessary. The findings provide a pathway for the creation of inhibitors with two functions; one might have antibacterial properties by inhibiting PBPs, while the other could be strong against metallo-β-lactamases and serine-lactamases.

Mechanism-Based β-Lactamase Inhibitors

Figure 1 shows that the irreversible hydrolysis of the β-lactam ring is catalyzed by SBLs and MBLs. via vastly different reaction paths. As a natural progression from the action mechanism of PBPs, SBLs use an important Ser residue for catalysis (Figure 1a). This residue's activated hydroxyl group is accountable for the nucleophilic assault on the β-lactam's amide bond. An intermediate tetrahedron is formed in this phase by using the sp3 hybridization of the carbon atom that is produced by the β-lactam. Figure 1a shows that this species forms as the active site's oxygen levels rise. A covalent link is formed between the Ser residue and the β-lactam's prior carbonyl carbon. An oxyanion hole, a positively charged slit on the active site, communicates with the tetrahedral intermediate, maintaining its negative charge. The next step is for an acyl-enzyme intermediate to be formed by the amide bond cleavage. In the last stage of the process, The hydrolyzed product is held to the enzyme by a cleaved and protonated covalent bond. At this point in the process, deacylation has reached its limit.

The first motivation for proposing potential behavior patterns for MBLs came from the mechanism of SBLs. Without the space for oxyanions and the catalytic Ser residue, this technique cannot be applied to MBLs. At now, there is a general consensus that the experimentally observed reaction consists of two primary steps: Figure 1b shows the nitrogen atom protonation, and the carbonyl being attacked by nucleophiles. What each metal ion does, how chemical intermediates build up, and how to find the nucleophile and proton donor are among the many controversial issues surrounding this general framework.
There is no MBL reaction scheme without the Zn(II) ions. Substrate positioning in the active site, nucleophile activation, stability of the numerous species created, and proton donor location are all dependent on these metal ions, as shown in Figure 1b. In Zn(II) hydrolases, the nucleophilic attack is enhanced when the pKa of a bound water molecule is lowered by a metal ion. Glyoxalase II, carboxypeptidase A, and carbonic anhydrase are all hydrolases. Some have suggested that Zn1 serves a comparable purpose for B1 and B3 MBLs. The absence of Zn1 in B2 MBLs makes Zn2 the only structurally maintained component of all MBLs; it is crucial for the reaction because Zn2 positions and stabilizes many species.

Despite their structural distinctions, the three subclasses of MBLs have a number of

commonalities in their response mechanism (Figure 1b). Upon binding to the substrate, the Michaelis complex interacts with residues around the metal site. Nucleophilic attack begins with the activation of a water/hydroxide molecule due to interactions with metal ions (B2.) or hydrogen bonding (B1. and B3). The non-trapped tetrahedral intermediate, which is different from SBLs, forms before the β-lactam ring cleavage and is energetically near the related transition states. The production and stability of anionic intermediates are outcomes of hydrolysis of carbapenems and cephalosporins, as seen in Figure 1b, c. These intermediates lack a tetrahedral carbon and are instead linked to the location where action takes place due to electrostatic interactions, since the β-lactam ring's C-N bond has already been broken. (Figure 1b). The antibiotic's structure after hydrolysis retains a strong interaction with Zn2, which delocalizes the negative charge. The rate-limiting step follows protonation. It culminates in the production of the final product and the subsequent restoring the unbound enzyme.

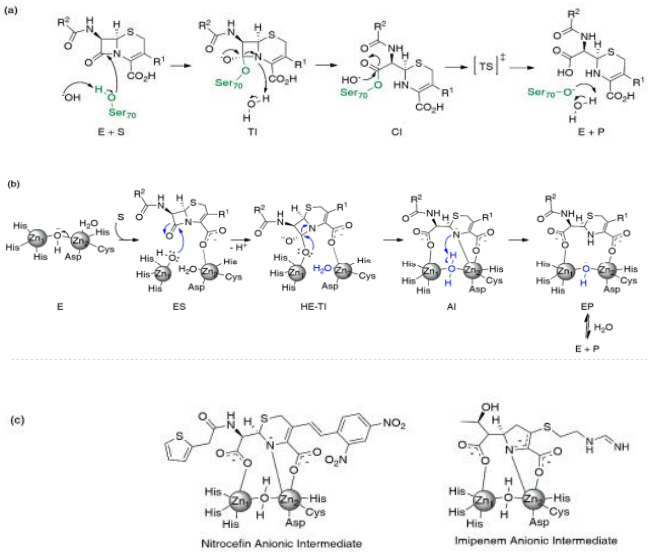

Figure 1. Reaction mechanism of serine-β-lactamases (SBLs) and MBLs.

Mechanism-Based Inhibitors of SBLs

In order for anti-β-lactamase drugs to be prescribed, they must be non-toxic and designed to effectively block β-lactamase enzymes. When used with β-lactam antibiotics, they must also have comparable pharmacokinetic features. Since the groundbreaking 1984 implementation of clavulanic acid-amoxicillin-ticarcillin as a combination, other SBLs inhibitors have been marketed. Shortly thereafter, two penicillanic acid sulfones, sulbactam and tazobactam, were developed. A number of penicillins and cephalosporins are used with these compounds for treating infections caused by bacteria that generate numerous class A SBL. These medications are unable to inhibit γ-lactamases belonging to classes B, C, and D, as well as KPC and other class A carbapenemases. They work by mimicking the chemical mechanism of SBLs, which are hydrolyzable owing to the presence of the catalytic Ser residue. An ineffective deacylation step causes these chemicals to covalently attach to the catalytic Ser residue, rendering the enzyme irreversibly inactive [80,82]. All of these chemicals are being considered potential suicide substrates based on mechanisms.

The diazabicyclo[3.2.1]octanone (DBO) functional group is present in the newly synthesized β-lactamase inhibitor Avibactam. Avibactam differs from the compounds mentioned before in that it lacks a β-lactam ring, is hydrolyzable, and has the ability to reversibly acylate SBL. Since its approval for clinical usage in 2015, the ceftazidime-avibactam combination has been effective against all subclasses of SBLs, including those that possess carbapenemase activity. Even while avibactam alone won't kill MBL-expressing Enterobacterales, when combined with aztreonam, it will kill both types of bacteria (since aztreonam is able to evade MBLs). We are now doing phase 3 clinical studies using this formulation.

Although research on boron-based inhibitors dates back to the 1980s, In 2017, the FDA authorized varofloxacin, the pioneering boron-based SBL inhibitor that made its way into clinical use, in combination with meropenem. Class D enzymes and MBLs are unaffected by this inhibitor, but classes A and C selective binding sites are successfully blocked. Such a covalent bond is reversible. is formed between the active Ser residue and the competitive inhibitor vaborbactam. Among the several boron-inhibitors and DBOs now undergoing clinical trials, relebactam stands head and shoulders above the others.

Difficulties in Developing a Selective MBL Inhibitor

Since no licensed SBL inhibitor is effective against MBLs, it is not possible to immediately apply the extensive information obtained about SBL inhibition to the development of MBL inhibitors. The primary reason for this is because the two kinds of β-lactamases, as previously stated, differ in their catalytic processes, the kind of nucleophile that attacks, aspects of the accumulating intermediate species in every case, as shown in Figure 1. A further distinction between SBLs and MBLs is the topology of their active sites. The active Ser residue is buried in a small but deeply placed catalytic site, is present at the domain junction in SBLs. On the other hand, MBLs include solvent-exposed Zn(II) ions near the base of a shallow groove where the active sites are positioned. Another issue is the

significant structural difference between MBLs and SBLs, which was already brought out. Distinct active site topologies, very little sequence homology among residues in the active site, and varying stoichiometries of zinc (II) are the three ways in which this variety is manifested.

Efforts to create MBL inhibitors using a broad range of methodologies have been documented in the literature. The structural diversity of MBLs has hindered the rational design. Many the established method of inhibiting zinc enzymes has led to the development of new chemicals; however, none of them have reached the clinic as of yet. These compounds include Sulfonamide compounds, hydroxamates, and thiol groups are only a few examples. Resources for searching chemical libraries, screening fragments, and doing virtual screenings using chemical libraries (both natural and synthesized), and microbe or plant extracts were used to find potential inhibitors**.** Even while several of these chemicals worked well against certain MBLs, they almost never achieved cross-class inhibition. Research into the interactions of these unique mechanistic species may lead leading to the creation of an inhibitor with a wide range of activity, as our earlier research demonstrated that the chemical mechanism underlying all MBLs remains consistent.

Most of these initiatives have been reviewed in good detail in a number of publications [8,120–126]. Here, we'd want to provide a new point of view by zeroing in on MBL inhibitors that get their inspiration from the ways in which the various species produced during catalysis interact with the enzymes. Substrate architectures and interactions between tetrahedral transition states will form the basis of our first inhibitor analysis. Then, we'll talk about those that are based on the Enzyme: Product (EP) structures, which are stable anionic intermediates. Since chelators remove the necessary Zn(II) ions, we shall quickly examine their role in MBL inhibition.

Anti-MBL Agents Derived from Substrate Structures

Conformational alterations are often seen during attaching to the substrate, the first step of the reaction. Interactions between β-lactam molecules and MBLs form the Michaelis complex (ES) has not been crystallized despite several experimental attempts. Substrate binding causes conformational changes in bi-metallated MBLs, according to results of L1 and BcII enzyme fluorescence experiments with stopped-flow. According to these findings, Zn(II) ions serve an important electrostatic anchoring function in substrate binding and are hence crucial. The effects on Michaelis complexes used the coordination sphere of the metal site studied via experiments with enzymes that were replaced with Co(II), as well as through experiments using stopped-flow and fast freeze quenching; X-ray absorption spectroscopy confirmed these results. Previous research has shown that Although interactions between metals and substrates are crucial for substrate binding, the roles played by the active sites L3 and L10 of B1 enzymes are equally significant. These findings from experiments, together with the plethora of crystal structures including EP complexes and several docking investigations, have led to the proposal of a generic substrate binding mechanism.

Figure 1 shows that all bicyclic β-lactam compounds contain a carboxylate group that acts as a binding force. This group coordinates directly involves Zn2 and a positively charged residue, often a Lys in B1 MBLs, at position 224, as shown in Figure 2a. Nevertheless, this interaction occurs with residue 2 in VIM enzymes. Near the bridging hydroxide of bi-Zn(II) enzymes is where you may find the β-lactam ring using this binding approach, which enhances the nucleophilic attack, as shown in Figure 1b. There are other interactions that are preserved with the nitrogen of Asn233 in the main chain (Figure 2a). Very few particular interactions have been observed, however the active site groove can accept the diverse substituents of the antibiotics. The reason why Aztreonam's binding to MBLs is ineffective is the β-lactam group is located distant from the nucleophile that is attacking it because sulfonate group comes into direct contact with Zn2, which stops hydrolysis.

The mono-metallated version of B2 MBLs is the most effective since they are exclusive carbapenemases. Substrate binding manner and catalytic process may be better understood by crystallographic examination of CphA bound to hydrolyzed biapenem, docking studies, and investigations of ImiS's intrinsic fluorescence. Secondary interactions between Lys224 and Val67 initiate carboxylate coordination to the metal ion. Hydrogen bonding interactions between His118 and Asp120 activate the attacking water molecule on metal-vacant site 1 in bi-metallated MBLs. This is the primary distinction from other MBL structures. The outcome is a less powerful nucleophile and ES complexes that are easier to investigate using spectroscopic methods, since they are more stable which are superior to those produced by bi-metallated enzymes. It has been suggested that a potential attachment location 1 for a second metal ion of the enzyme, rendering it inactive and perhaps displacing the nucleophile. An further mechanism that helps dissolve amide bonds is the interaction between nitrogen and zinc(II) ions.

It is believed that all MBLs undergo a nucleophilic attack just before Figure 1b shows that a high-energy tetrahedral intermediate (HE-TI) forms when

the C-N bond breaks. The experimental feature of this high energy state is lacking; however, it has some similarities to the tetrahedral intermediate (TI, Figure 1a) that has been suggested for SBLs. Assuming the carboxylate group and Zn2 site remain in contact, Sp3 carbon synthesis close to the Zn1 site is the distinguishing characteristic.

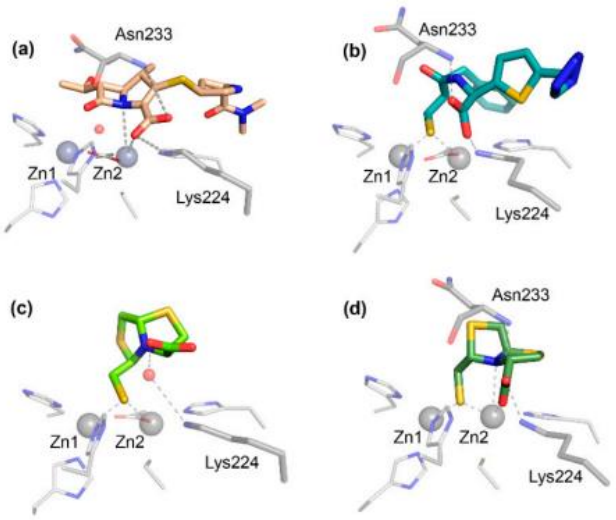

Figure 2. Binding modes of substrate mimic inhibitors.

Substrate Mimic Inhibitors

It is believed that compounds imitating the structures or the modalities of interaction will be effective inhibitors of the ES complex since its production requires unique interactions between substrates and enzymes. This approach allows for the removal of most β-lactam substrates since the majority of MBLs are broad-spectrum enzymes development of molecules that have the potential to bind to several MBLs. Several compounds based on the structure of the β-lactam antibiotic will be examined in this section.

The first inhibitor of MBL substrate mimics, mercaptocarboxylate 1, is shown in Figure 3. This molecule has many chemical components with benzylpenicillin, including a carboxylate, an aromatic group that stands in for the R1 chain, and a linear amide. The ornamentation is finished by adding a thiol, which is a common zinc binding group. The medication inhibited Table 1 shows the nM range for IMP-1 and L1. Within the nM range includes the commonly used half-maximal inhibitory doses (IC50) or inhibition constants (Ki) below 90 nM. At rest, the enzyme loses its catalytic water because the thiol binds to its metal core, which connects the two Zn(II) ions. The remaining portion of the molecule exhibits hydrophobic interactions with L3 and interactions with important residues like Asn233 and Lys224 (Figure 4b). Although these properties mirror those of the substrates, the binding and inhibition are caused by the strong interaction between Zn(II) and thiolates. The active site contains the remainder of the inhibitor, which forms hydrogen bonds that are different from the substrates.

Bicyclic substrate mimics have been developed using several ways. A component of β-lactams is the incorporation of various groups onto their bicyclic core. Figure 3 depicts the screening technique that was applied to a set of 1β-methylcarbapenem derivatives was used to find J-110,441 2, as mentioned in the reference. Table 1 shows that adding a benzothiophene group to the C2 position of B1 and B3 MBLs as well as SBLs from A and C courses produced strong inhibitory effects in the region of low microMolar Ki values. This range includes potencies that are used often, ranging from 9 microM to 0.1 microM. Inhibition potencies were low due to spacer inclusion, identical side chain replacement at position 3, or substitution of amides for certain methines. 2 made clinical isolates more sensitive to ceftazidime or imipenem using the cephalosporin scaffold, Buynak et al. explored the potential of constructing a reverse hydroxamate by swapping N-OH for N-H at C7, which would allow bound to the active site are the Zn(II) ions. In tests conducted against B1 enzymes, the third derivative that proved to be the most potent had an IC50 value in the low microM range, as shown in Table 1.

The penicillin scaffold was modified to develop dual SBL and MBL inhibitors to produce a stable acyl-enzyme complex that inhibits SBLs and a thiol group that targets MBLs. Figure 5 shows that compound 4, which was one of the compounds in Table 1, Having an IC50 value that falls within the low microM range when tested against BcII, L1, TEM-1, and P99. The minimum inhibitory concentrations (MICs) for MBLs were reduced when piperacillin was added to four several bacterial strains, such as Escherichia coli IMP-1 and Pseudomonas aeruginosa carrying VIM or SPM-1.

One technique to mimic substrates is to make β-lactam antibiotics less reactive to hydrolysis without compromising their binding properties to the active site. A thioamide is used in place of the β-lactam amide in 8-thioxocephalosporins, which changes the compounds' acidity, charge distribution, and reactivity. As anticipated, 8-thioxocephalosporin 5a (Figure 3) exhibited mild inhibitory action (Table 1) and was weakly hydrolyzed by BcII. The thioacid 5b, which was a byproduct of hydrolysis (Figure 3), had a more potent inhibitory action on BcII (Ki = 96 µM). The mono-thioxo-piperazinedione 5c is formed when the thioxocephalexin in solution undergoes intramolecular aminolysis (Figure 5). This compound has a higher efficacy in inhibiting BcII (Ki = 29 µM).

In Figure 3, you can see the bisthiazolidine scaffold, which was created to resemble the structure of

penicillins. Its metal binding group is a thiol, however, rather than an amide. The following enzymes were shown to be efficient inhibitors of MBLs: B1 A2, B3 (Sfh-I), BcII, NDM-1, IMP-1, and VIM-2 (LOB-18, B3). Because of its reduced active site, Sfh-I's inhibitory effectiveness was impacted by the stereochemistry of its chiral centers. Figure 2c,d demonstrates that conserved B1 subclass residues such Asn233 and Lys224 are bound by the carboxylate group, and that the metal center is contacted by the thiol group to control the binding of bisthiazolidines to binuclear B1 and B3 enzymes. The outcome is that these compounds can accommodate the active site of MBLs and exhibit flexible binding behavior. The β-lactam antibacterial action was restored in the presence of the most powerful inhibitor, L-6 many clinical strains that produce MBL (Figure 3 and Table 1). Neither eukaryotic cells nor the human metalloenzyme glyoxalase II, which has an MBL-related protein fold, were inhibited or harmed by L-6.

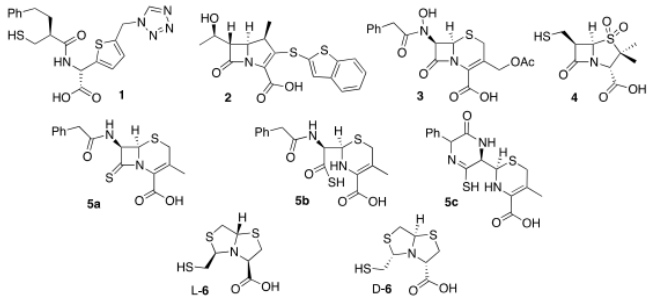

Figure 3. MBL substrate mimic inhibitors.

CONCLUSION

Substrate structures were used as inhibitor templates in the early years of MBL research because there are no readily available crystal structures. With the advent of modern tools like libraries and displays that employ fragments, a large number of compounds were found that were completely unrelated to the catalytic species. A large portion of them were only engaged with a small number of MBLs from the same subclass. There were new ways to approach chemical synthesis when the catalytic mechanism was discovered. This is because boron-based compounds may inhibit all forms of β-lactamases and match the common features of the tetrahedral intermediate in SBLs and the tetrahedral transition state in MBLs, making them very intriguing chemicals. Adding boron to tangiborbactam, the first MBL inhibitor to get FDA approval, is very probable. One strategy to use the avidity of thiol moieties towards Zn(II) is to make prodrugs with the thiol group protected so that it may be released inside the cell. One approach to harnessing the affinity of thiol groups for zinc is to create prodrugs with the thiol group protected, allowing their release inside the cell.

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