Abstract
The production of metallo-β-lactamases is the most important strategy by which pathogenic bacteria become resistant to currently known β-lactam antibiotics. The emergence of these enzymes is particularly concerning for the future treatment of bacterial infections. There are no clinically available drugs capable of inhibiting any of the metallo-β-lactamases, so there is an urgent need to find such inhibitors. In this review, an up-to-date status of the inhibitors investigated for the inhibition of metallo-β-lactamases has been given so that this rich source of structural information of presently known metallo-β-lactamases could be helpful in generating a broad-spectrum potent inhibitor of metallo-β-lactamases.

Keywords
Metallo-beta-lactamases • Inhibition • Thiol • β-Lactam analogues • Peptides • Biphenyl tetrazoles • Triazoles

Introduction
β-Lactam antibiotics are the most widely used antibacterial agents for treating bacterial infections. However, many pathogenic bacteria have developed resistance against these antibiotics through mechanisms such as a decrease in cell wall permeability, efflux pump, and hydrolysis of the β-lactam ring by β-lactamases [1, 2]. β-Lactamases of class B are metallo-proteins, also called metallo-β-lactamases. These enzymes use a zinc-bound hydroxyl group as the nucleophile [3] to promote the hydrolysis of a very broad range of β-lactam antibiotics, including penicillins, cephalosporins, and carbapenems [4].
Serine-β-lactamase inhibitors do not inhibit metallo-β-lactamases and there are no clinically approved inhibitors of metallo-β-lactamases. So there is an urgent need for the development of such an inhibitor that could inhibit all metallo-β-lactamases. Here, we discuss currently available MBL inhibitors from different groups to provide a collective view of the chemical structures of these inhibitors, which could be helpful in designing inhibitors capable of meeting the serious biological threat, resistance of pathogenic bacteria to β-lactam antibiotics.

**Phenazines**

Gilpin et al., [5] isolated two novel phenazines (SB 212021 and SB212305) from a streptomyces in 1995 and screened them against three metallo-β-lactamases Xanthomonas maltophilia L-1, Bacteroides fragilis 262 CfiA, and Bacillus cereus II. These compounds are non-specific and appear to chelate the zinc, so when zinc levels are increased, enzyme activity recovers. In the absence of added zinc, both compounds had IC50s of 1~75 µM for the B. fragilis 262 CfiA and X. maltophilia L-1 metallo-β-lactamases. SB212305, which contains a thio group, has the lowest IC50 = 1 µM for X. maltophilia L-1, while in the case of B. cereus II, the phenazine SB212021 has the lowest IC50 = 37 µM (Figure 1).

![Phenazine inhibitors](image)

**Thiol derivatives**

Due to the high affinity of sulfur for zinc, it has been found that compounds having a thiol group showed promising inhibition against MBLs. A series of mercaptoacetic acid thiol esters [6] was synthesized and identified as metallo-β-lactamase inhibitors. Mass spectrometric data suggest that mercaptoacetic thiol esters act as mechanism-based inhibitors for BcII by generating mercaptoacetic acid in situ, which forms a disulfide linkage with a cysteine residue in the active site of the enzyme. The inhibitors of this series showed a broad range of potencies (IC50’s varied from mM to µM) against the enzymes.

A series of thioesters and thiols synthesized by a novel solid-phase Mitsunobu reaction were screened [7] against the CcrA and IMP-1 enzymes. These compounds showed better inhibition for IMP-1 than for CcrA MBL. In the thiodepsipeptides series, the most potent inhibitor was compound 1 with an IC50 of 0.25 µM against IMP-1. However, the thiol moieties of the thiodepsipeptides showed better inhibition, with IC50s of 0.086–0.023 µM against IMP-1. On the other hand, the simple acetyl and benzoyl thioesters were found to
be significantly more potent inhibitors than the thiols themselves. This comparison of activity of thiols and thioesters is shown in the Figure 2.

![Chemical structures](image)

**Fig. 2.** Comparison of IC$_{50}$ values of thiols and thiol esters

In 1998, S. Bounaga and his co-workers [8] reported N-(2'-mercaptoethyl)-2-phenylacetamide 6 as a competitive inhibitor of β-lactamase II from *B. cereus* with a $K_i$ of 70 mM. This compound was also identified as a competitive inhibitor of L1 with a $K_i$ of $50 \pm 3$ µM [9].

Thiomandelic acid [10] was identified as a broad spectrum inhibitor with a sub-micromolar $K_i$ value for the nine MBLs tested, except that from the *Aeromonas hydrophila* enzyme. The $K_i$ values of R and S-thiomandelic acids for *B. cereus* enzyme were found 0.09 and 1.28 µM, respectively (Figure 3). Kurosaki et al., [11] reported the two irreversible inhibitors 7 and 8 with $K_i$ values of $3.452 \pm 0.030$ µM and $0.423 \pm 0.013$ µM, respectively for the IMP-1 enzyme (Figure 3).

Siemann et al., also studied thiols as classical inhibitors of MBLs and reported mercaptoacetic acid 9 (IC$_{50}$ = 1.3 µM) and 1,2-benzenedimethanethiol 10 (IC$_{50}$ = 2.3 µM) as inhibitors of the IMP-1 enzyme [12]. Demonstrating the efficiency of the Dynamic Combinatorial Mass Spectrometry (DCMS) technique, N-Benzoyl-D-cysteine 11 [13] was identified as the potent inhibitor for Bcll enzyme with a $K_i$ value of 740 nM. D-captopril [14] was reported as a broad spectrum potent inhibitor of subclass B1 and B3, but its potency against subclass B2 is low ($K_i = 72$ µM).

In search of potent inhibitors of all subclasses of MBLs, Liénard and his co-workers [15] synthesized compounds containing thiol function(s). Compounds 12 and 13 were found to inhibit MBLs from all three subclasses. For the monozinc CphA MBL, compounds 12 and 13 were reported to be the most potent inhibitors with $K_i = 90$ nM and $K_i = 50$ nM, respectively. Lassaux et al., [16] synthesized mercaptophosphonate compounds and screened them against all subclasses of MBLs. Most of their synthesized compounds were found to be competitive inhibitors for all three subclasses B1, B2, and B3 MBLs. With some exceptions, all the mercaptophosphonate derivatives showed good inhibitory effects
on the CphA, a subclass B2 enzyme with low inhibition constants ($K_i < 15 \mu M$). The most potent broad spectrum inhibitor 14 of this series is given in Figure 3.

The mercaptocarboxylate, PhenylC3SH (Figure 3) [17], acts as a potent inhibitor of the VIM-2 enzyme with a $K_i$ value of 220 nM, whereas it is less active for the IMP-1 enzyme ($K_i = 1660 \text{ nM}$) [18].

![Chemical structures of compounds 6-15]

**Fig. 3.** Thiols inhibitors of MBLs

Concha and others [19] determined the crystal structure of the IMP-1- mercaptocarboxylate 15 complex and found that this mercaptocarboxylate inhibits the IMP-1, *B. fragilis*, and L1 enzymes with IC$_{50}$ values between 100 and 500 nM. W. Jin [18] and his colleagues studied the inhibitory effect of two series of compounds, 2-$\omega$-phenylalkyl-3-mercaptopropionic acids and N-[(7-chloro-quinolin-4-ylamino)-alkyl]-3-mercaptopropionamides, on IMP-1 and VIM-2 metallo-ß-lactamases. Among the first series, PhenylC4SH (Figure 3) was found to be the potent inhibitor of both IMP-1 and VIM-2 with IC$_{50}$ values of
1.2 and 1.1 µM, respectively, whereas QuinolineC4SH (Figure 3) of the second series showed maximum inhibition for the IMP-1 (IC\textsubscript{50} = 2.5 µM) and VIM-2 (IC\textsubscript{50} = 2.4 µM) enzymes.

**Trifluoromethyl alcohols and ketones**

Trifluoromethyl ketones are reported as serine proteases [20, 21] and zinc dependent carboxypeptidase [22] enzymes. Walter et al., [23, 24] reported the trifluoromethyl compounds as the first synthetic inhibitors of different strains of MBLs. They synthesized different N-phenoxyacetyl-substituted trifluoromethyl ketones and alcohols and screened against MBLs from *X. maltophilia* ULA-511, *A. hydrophila* AE036, *B. cereus* 569H, and *Pseudomonas aeruginosa* 101 as inhibitors. Among these trifluoromethyl ketones and alcohols, the most potent inhibitors 16–19 are given in Figure 4 along with the $K_i$ values.

\[
\begin{align*}
16 & \quad K_i = 30 \text{ µM (for B. cereus)} \\
17 & \quad K_i = 60 \text{ µM (for P. aeruginosa)} \\
18 & \quad K_i = 3.0 \pm 0.4 \text{ µM (for X. maltophilia)} \\
19 & \quad K_i = 15 \pm 1 \text{ µM (for X. maltophilia)}
\end{align*}
\]

**Fig. 4.** $K_i$ values of trifluoromethyl ketones and alcohol

**Biphenyl tetrazoles**

Screening of the Merck chemical collection by Toney et al., [25] led to the identification of biphenyl tetrazoles as potent competitive inhibitors of metallo-β-lactamase (*B. fragilis*). The structure-activity relationships of biphenyl tetrazoles showed that unsubstituted BPT is a poor inhibitor with IC\textsubscript{50} value of 860 ± 60 µM. It was found that the ortho position of the tetrazole group, relative to the biphenyl ring system, is important in enzyme inhibition, because movement of the tetrazole group to the meta or para positions led to the loss of inhibitory activity [25]. It was also found that substitution at the 4'-position of the BPT further increased the inhibitory activity of the BPTs. Figure 5 shows the IC\textsubscript{50} values comparison of the parent BPT and the substituted BPTs. To further explore the potency of BPTs as MBLs inhibitors, in 1999, Toney et al., [26] screened a series of BPTs containing 3-n-butyl-1-phenylpyrazole-5-carboxylate against *B. fragilis* and IMP-1 metallo-β-lactamases. The parent BPT 20 was found to be a weak inhibitor with an IC\textsubscript{50} of ~ 200 ± 8
µM of *B. fragilis* MBL, while against IMP-1, it was found to be inactive (IC$_{50}$ >200 µM). The substitution upon the phenyl ring and esterification of the carboxylic group of compound 20 lowered the IC$_{50}$ value up to 60 ± 30 µM 21 for IMP-1 and 18 ± 2 µM 22 for *B. fragilis* MBL (Figure 5).

![Chemical structures](image)

**Fig. 5.** Comparison of IC$_{50}$ values of BPT and substituted BPTs

**Succinic and phthalic acid derivatives**

The IMP-1 enzyme is a plasmid-borne zinc metalloenzyme responsible for the hydrolysis of β-lactam antibiotics, including carbapenems, rendering them ineffective. To protect the broad spectrum antibiotics from hydrolysis by IMP-1, Toney *et al.* [27] have identified a series of 2,3-(*S,S*)-disubstituted succinic acids as potent inhibitors of IMP-1. Among this series, the most potent inhibitor is 23 with an IC$_{50}$ of 0.0027 µM. In 2005, Toney [28] and others reported several novel succinic acid derivatives, as IMP-1 inhibitors showing mixed inhibition. They reported compound 20707 with lowest $K_i$ value of 3.3 ± 1.7 µM.
Using docking methodologies and experimental enzyme kinetics, Olsen [29] and his coworkers identified several succinic acid derivatives as the di-zinc metallo-β-lactamase inhibitors. The potent inhibitors, 24 and 25 (Figure 6), of this series have an IC$_{50}$ value range of 10-100 µM. Hiraiwa et al., [30] synthesized substituted phthalic acids and screened against the IMP-1 enzyme for inhibitory activity. Phthalic acid has almost no inhibitory activity against IMP-1, but 3-substituted phthalic acid derivatives are potent inhibitors of this enzyme. Some of the most potent inhibitors 26–29 of this series, along with their IC$_{50}$ values, are given in Figure 6.

**Fig. 6.** Succinic and phthalic acid derivatives as MBL inhibitors

### Hydroxamates

Walter et al., [31] synthesized amino acid-derived hydroxamates and screened against different MBLs for inhibitory activity and found several compounds as the inhibitors of clinically relevant enzymes from *A. hydrophila*. In 2006, B. M. R. Liénard and his colleagues [32] synthesized a series of derivatives of benzohydroxamic acid 30 and tested them against FEZ-1, IMP-1, BcII, CphA, and L1 MBLs for inhibitory activity. Their study resulted in the identification of selective inhibitors of FEZ-1 metallo-β-lactamase. The most potent selective inhibitor of FEZ-1 from this series was identified as 2,5-substituted benzophenone hydroxamic acid 31 (Figure 7) with a $K_i$ value of 6.1 ± 0.7 µM.
In search of broad spectrum potent inhibitors of MBLs, a variety of 1β-methylcarbapenem conjugates were tested against different MBLs [33]. The kinetic studies showed that 1β-methylcarbapenems having dithiocarbamate, benzothienythio, or pyrrolidinylthio moieties at the C-2 position showed promising inhibition against MBLs. The most potent inhibitor among these compounds is J-110,441, which simultaneously targets class A, B, and C β-lactamases. It almost acts as a broad spectrum inhibitor of MBLs with $K_i$ values of 0.83, 1.00, 0.23, and 0.0037 µM for II from *B. cereus*, L1 from *Stenotrophomonas maltophilia*, CcrA from *B. fragilis*, and IMP-1 MBLs, respectively [33]. A novel 1β-methylcarbapenem with a trans-3,5-disubstituted pyrrolidinylthio moiety at the C-2 position (J-111,225) inhibits the IMP-1 enzyme with a $K_i$ of 0.18 µM [34]. F. V. Hovel et al., [35] also reported penicillin and their rearranged products as potential inhibitors of *B. cereus* MBL.

Buynak et al., [36] synthesized penicillin-derived inhibitors that simultaneously inhibit both serine and metallo-β-lactamases. The 6-(mercaptomethyl)penicillinates 32–35 (Figure 8) were found as good inhibitors of L1 and BCI1 MBLs with a $K_i$ range 0.10–32.1 µM. Tsang et al., [37] reported 8-thioxocephalosporins as weak competitive inhibitors ($K_i \sim 700$ µM) of *B. cereus* MBL. Interestingly, the hydrolysis product of thioxocephalexin, a thioacid, acts as a competitive inhibitor with a $K_i = 96$ µM, while the cyclic thioxo-piperazinedione, formed by intramolecular aminolysis of thioxo-cephalexin, inhibits the same enzyme with a $K_i$ of 29 µM. Badarau [38] and his coworkers also reported that the hydrolysis products of cephalosporins and thiols inhibit the *B. cereus* MBL at the micromolar range. Beharry et al., [39] identified 6-alkylidene-2-substituted penam sulfoxes as inhibitors of Bla2 with IC$_{50}$ values less than 10 µM. Compound 36 is the representative of this series with the lowest IC$_{50}$ of 1.0 µM. A series of cephalosporin-derived reverse hydroxamates and oximes were prepared and tested against MBLs for inhibitory activity. The reverse hydroxamates were found to inhibit the GIM-1 MBL at the submicromolar level [40]. Cyclobutanone analogues of β-lactams [41] (37 and 38) were also reported as the inhibitors of the IMP-1 enzyme.
Peptides

Sanschagrin et al., [42] reported a peptide as an inhibitor for metallo-β-lactamases. Cys-Val-His-Ser-Pro-Asn-Arg-Glu-Cys was identified as a promising inhibitor of the L1 enzyme showing mixed inhibition, $K_i$ competitive of 16 ± 4 µM and a $K_i$ uncompetitive of 9 ± 1µM.

Bounaga et al., [23] synthesized several cysteinyl peptides and identified N-carbobenzoxy-D-cysteinyl-D-phenylalanine 39 as the most potent reversible competitive inhibitor of the B. cereus MBL with a $K_i$ value of 3.0 µM. A library of homo-cysteinyl peptides [44] was synthesized and screened for inhibitory activity against L1 metallo-β-lactamase. It was found that homo-cysteinyl peptides are more active than the cysteinyl peptides. The most active compound of the homo-cysteinyl peptides is 40 with a $K_i$ value of 2.1 nM (Figure 9).

Pyridine Dicarboxylates

Different pyridine dicarboxylates were tested against different MBLs. 2-picolinic and pyridine-2,4-dicarboxylic acids 41 and 42 (Figure 9) were identified as competitive inhibitors of CphA MBL with $K_i$ values of 5.7 and 4.5 µM, respectively [45]. Roll et al., [46] screened natural products, pyridine monothiocarboxylic acid analogues, for inhibitory activity against MBLs. Among these naturally isolated compounds, dithioacid 43 (Figure 9) was found to be the strongest inhibitor of CcrA from B. fragilis and L1 from S. maltophilia.
Fig. 9. Cysteinyl peptides and pyridine dicarboxylic acid inhibitors of MBLs

Natural Products
Screening of an extract from a strain of Chaetomium funicola against B. cereus II resulted in the identification of tricyclic natural products (SB238569, SB236050, and SB236049) [47] (Figure 10) as MBL inhibitors. The most active of these natural products was the SB238569 with $K_i$ values of 3.4, 17.0, and 79.0 µM for B. fragilis CfiA, P. aeruginosa IMP-1, and B. cereus II MBL, respectively. The flavonoids galangin and quercetin [48] (Figure 10) were also reported as the inhibitors of MBL from S. maltophilia.

Fig. 10. Natural product-based inhibitors of MBLs

Triazoles and N-acylated thiosemicarbazides
The VIM-2 enzyme is the most commonly found MBL in clinical isolates worldwide [49–51]. In search of a potent inhibitor of VIM-2, Minond [52] and others screened a library of
pharmacologically active compounds and identified two potent and competitive novel sulphonyl-triazoles, 44 ($K_i = 0.41 \pm 0.03 \mu M$) and 45 ($K_i = 1.4 \pm 0.10 \mu M$), which are inhibitors of VIM-2 MBL. To improve the potency of compound 44, Weide et al., [53] varied the substitutions on the triazole ring and generated the most potent inhibitor 46 of this series with a $K_i$ of 0.01 ± 0.001 µM. Some other inhibitors 47–49 resulting from this work are given in Figure 11.

Vella et al., [2] recently screened a 500 compound Maybridge™ library for several new classes of leading inhibitors against the IMP-1 MBL, and considered the 4-methyl-5-(trifluoromethyl)-4H-1,2,4-triazole-3-thiol 50 ($K_i = 0.97 \pm 0.60 \text{ mM}$) the most promising for further study. We elaborated this ring system to increase the potency of this compound and identified the mercapto triazole 51 as showing mixed inhibition ($K_i$ competitive = 75 ± 30 µM and $K_i$ uncompetitive = 56 ± 10 µM) for the IMP-1 enzyme. We found that the N-acylated thiosemicarbazide intermediates in the synthesis of mercapto triazoles are also potent inhibitors of IMP-1 MBL with $K_i$ values as low as 11µM [54]. N-acylated thiosemicarbazide 52 has the maximum inhibitory activity showing mixed inhibition ($K_i$ competitive = 11 ± 4 µM and $K_i$ uncompetitive = 20 ± 5 µM) for the IMP-1 enzyme Figure 11.

Fig. 11. Triazoles and N-acylated thiosemicarbazide inhibitors of VIM-2 and IMP-1 MBLs
Tamilselvi and Mugesh [55] studied the hydrolysis of cephalosporins and found that cephalosporins having heterocyclic -SR side chains are less prone to MBL-mediated hydrolysis. This is partly due to the inhibitory activity of the thione moieties eliminated during hydrolysis. They carried out the enzymatic hydrolysis of oxacillin in the presence of heterocyclic thiones and disulfides 53–58 (Figure 12) and found that the catalytic activity of the Bcll enzyme was significantly reduced by these compounds. However, these inhibitors are not as potent as the aliphatic thiol 9 [12].

**Pyrrole Derivatives**

Mohamed et al., [56] recently synthesized pyrrole derivatives and tested for their inhibitory activity against the IMP-1 enzyme from *P. aeruginosa* and *Klebsiella pneumonia*. They reported some compounds showing micromolar inhibition constants for IMP-1 MBL. Compound 59 showed the maximum inhibition with a $K_i$ value of $12 \pm 4 \, \mu$M, while compounds 60 and 61 (Figure 13) have $K_i$ values of $15 \pm 5 \, \mu$M and $18 \pm 9 \, \mu$M, respectively.
Single Stranded DNAs

Kim [57] and his colleagues identified single-stranded DNAs as rapid, reversible, and non-competitive inhibitors of *Bacillus cereus* 5/B/6 MBL, with $K_i$ and $K_i'$ values in the nanomolar range. They assayed these ssDNA’s against other zinc-dependent metallo-enzymes like porcine carboxypeptidase A, but there was no inhibitory effect on the catalytic activity of these enzymes. Hence, these inhibitors are highly specific to *B. cereus* 5/B/6 metallo-β-lactamase. They found 30 residue ssDNA ($K_i = 0.92$ nM and $K_i' = 11$ nM) and 10 residue ssDNA ($K_i = 0.31$ nM and $K_i' = 1.5$ nM), the most potent non-competitive inhibitors of *B. cereus* MBL.

Sulfonic Acid Derivatives

Siemann [58] and his coworkers reported N-arylsulfonyl hydrazones as novel inhibitors of the IMP-1 enzyme. The most potent inhibitor of IMP-1 MBL is compound 62 with an $IC_{50}$ of 1.6 µM. 4-Morpholinoethanesulfonic acid 63 has also been reported as a competitive inhibitor of MBL from *B. fragilis* with a $K_i$ of 23 ± 5 mM [59]. Simm [60] reported bulgecin A 64 as a novel inhibitor of binuclear MBLs. It competitively inhibits ($K_i = 230 ± 10$ µM) BcII enzyme in its two-zinc form, but fails to inhibit when the enzyme is in the single-zinc form, while it inhibits ($K_i = 2.5 ± 0.3$ µM) the L1 enzyme in a partially competitive mode (Fig. 14).

![Fig. 14. Sulfonated inhibitors of MBLs](image)

Summary

To overcome the problem of increasing resistance of pathogenic bacteria by expressing metallo-β-lactamases to the presently known β-lactam antibacterial agents, several research groups are actively engaged in discovering broad spectrum potent inhibitors of metallo-β-lactamases. Several chemical classes of metallo-β-lactamases have been
reported, but there is no such inhibitor to overcome this problem. The challenge for the medicinal chemists and pharmaceutical industries will be to continue to identify such a broad spectrum inhibitor to get rid of this clinical threat. In this review, currently known potent inhibitors of metallo-β-lactamases are presented and we are hopeful that this review could provide a platform for designing a broad spectrum potent inhibitor of all metallo-β-lactamases.

Authors’ Statement

Competing Interests

The authors declare no conflict of interests.

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