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Review

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An Update on the Status of Potent Inhibitors of Metallo-β-Lactamases

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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-l, *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 IC_{50}s 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 IC_{50} = 1 µM for *X. maltophilia* L-1, while in the case of *B. cereus* II, the phenazine SB212021 has the lowest IC_{50} = 37 µM (Figure 1).

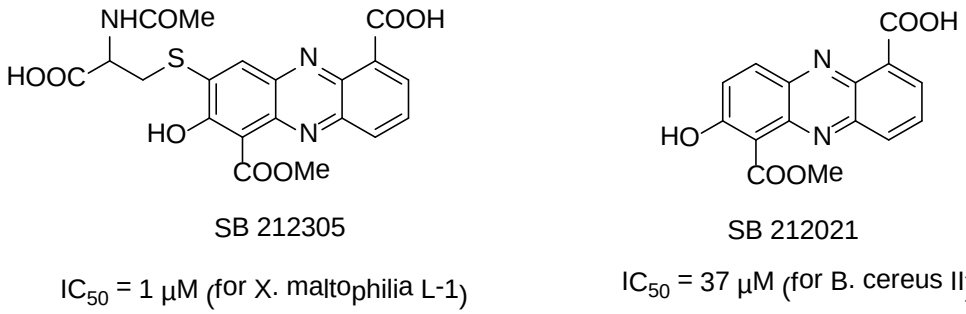


Fig. 1. Phenazine inhibitors

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 (IC_{50}'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 IC_{50} of 0.25 µM against IMP-1. However, the thiol moieties of the thiodepsipeptides showed better inhibition, with IC_{50}s 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.

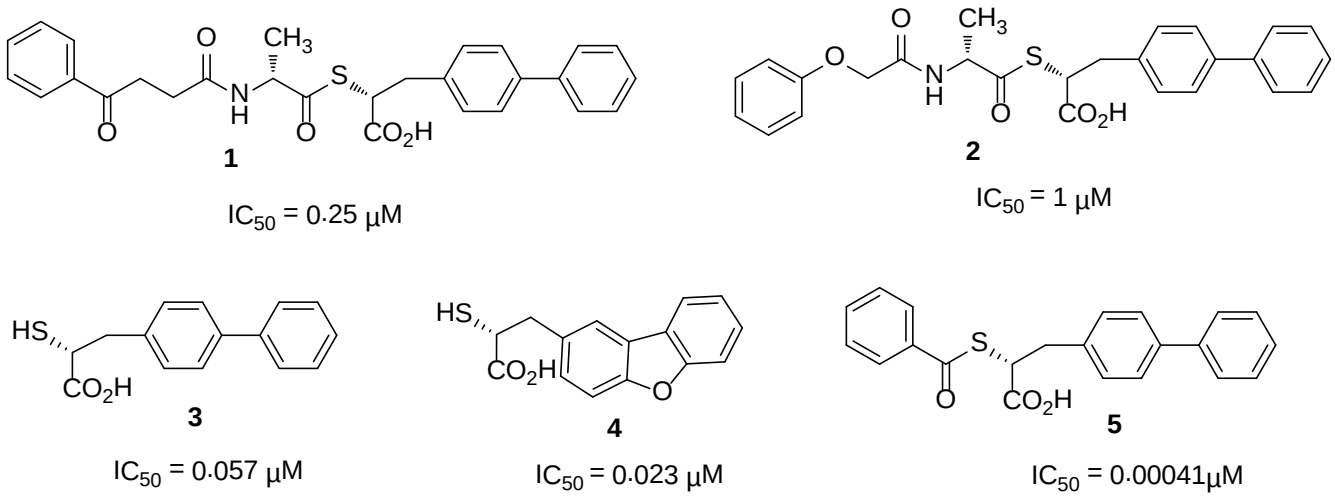


Fig. 2. Comparison of IC_{50} values of thiols and thiol esters

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 ± 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 *at al*., [11] reported the two irreversible inhibitors **7** and **8** with K_i values of 3.452 ± 0.030 μM and 0.423 ± 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 BcII 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 μM). 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 nM) [18].

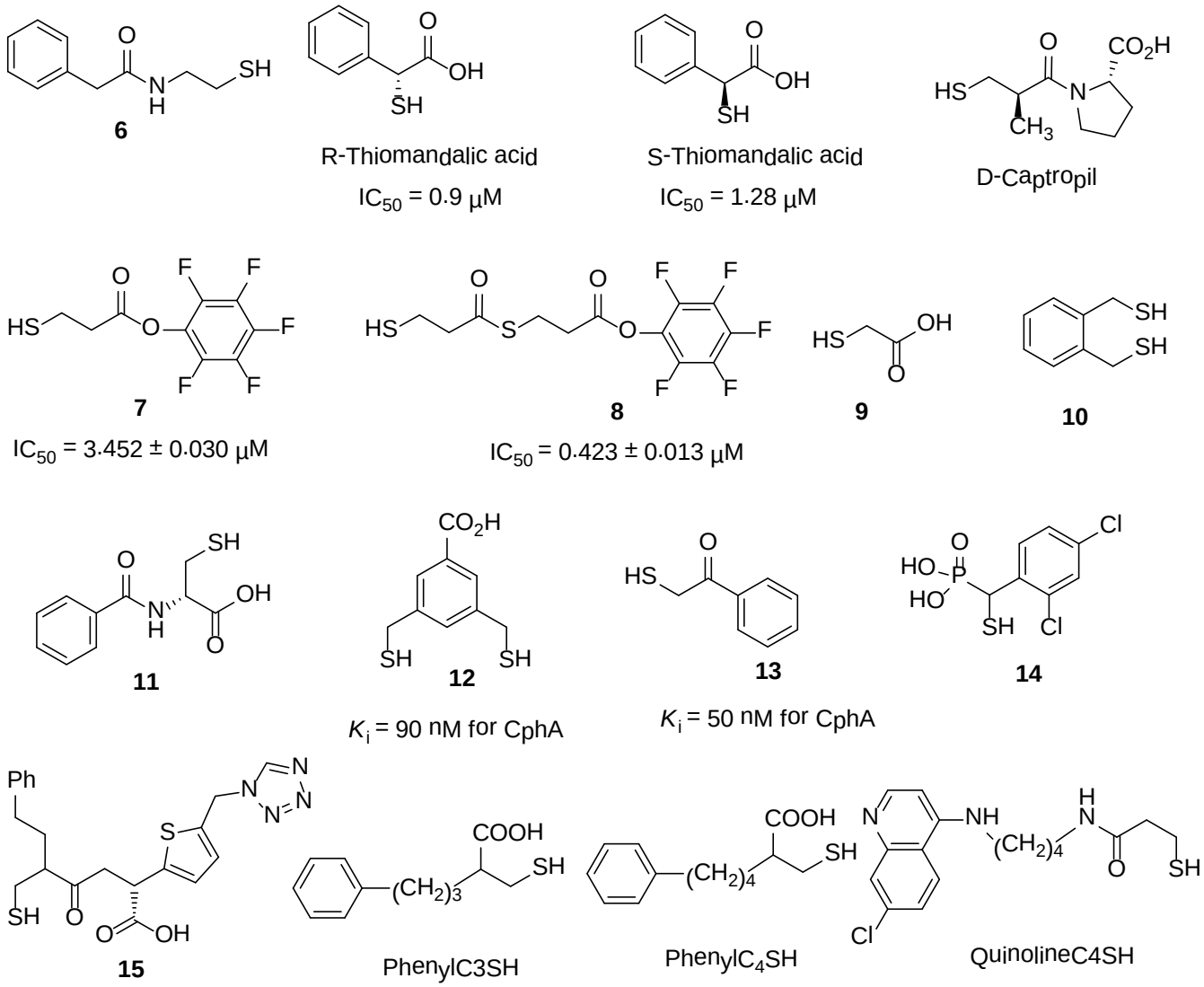


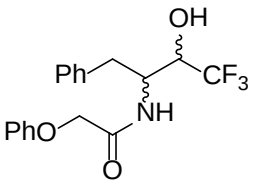
Fig. 3. Thiols inhibitors of MBLs

Concha and others [19] determined the crystal structure of the IMP-1- mercapto-carboxylate **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-ω-phenylalkyl-3-mercaptopropionic acids and N-[(7-chloro-quinolin-4-ylamino)-alkyl]-3-mercapto-propion-amides, on IMP-1 and VIM-2 metallo-β-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_{50} = 2.5 μM) and VIM-2 (IC_{50} = 2.4 μM) enzymes.

Trifluoromethyl alcohols and ketones

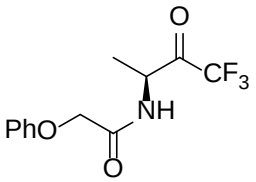
Trifluoromethyl ketones are reported as serine proteases [20, 21] and zinc dependent carboxypeptidase [22] enzymes. Walter *et al*., [23, 24] reported the trifluoromethyl compounds as the first synthetic inhibitors of different strains of MBLs. They synthesized different *N*-phenoxyacetyl-substituted 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.



16

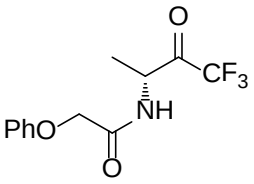
K_i = 30 μM (for B. cereus)

K_i = 60 μM (for P. aeruginosa)



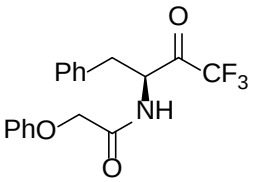
17

K_i = 1.5 ± 0.01 μM (for X. maltophilia)



18

K_i = 3.0 ± 0.4 μM (for X. maltophilia)



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K_i = 15 ± 1 μM (for X. maltophilia)

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_{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_{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_{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).

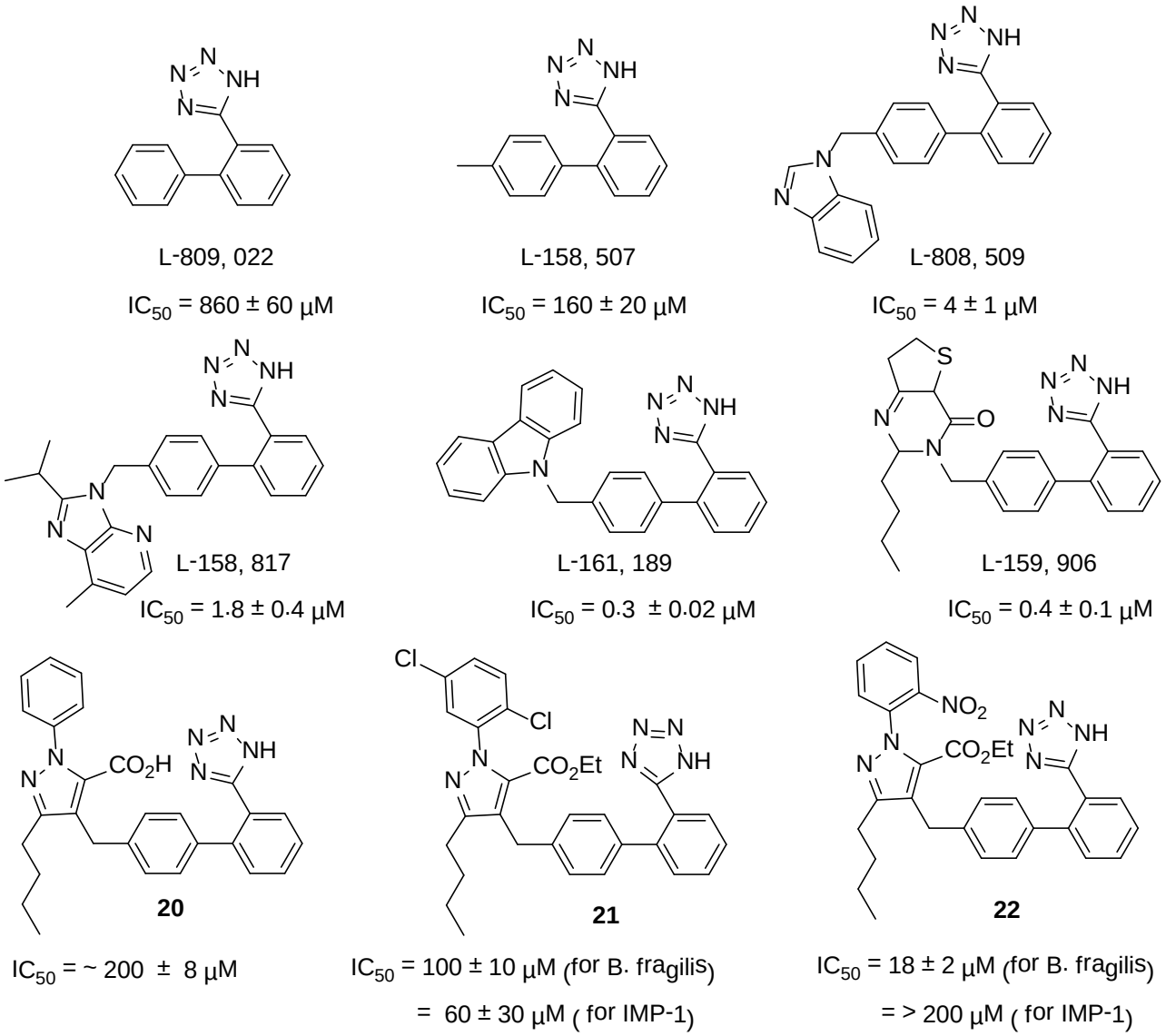


Fig. 5. Comparison of IC_{50} values of BPT and substituted BPTs

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.

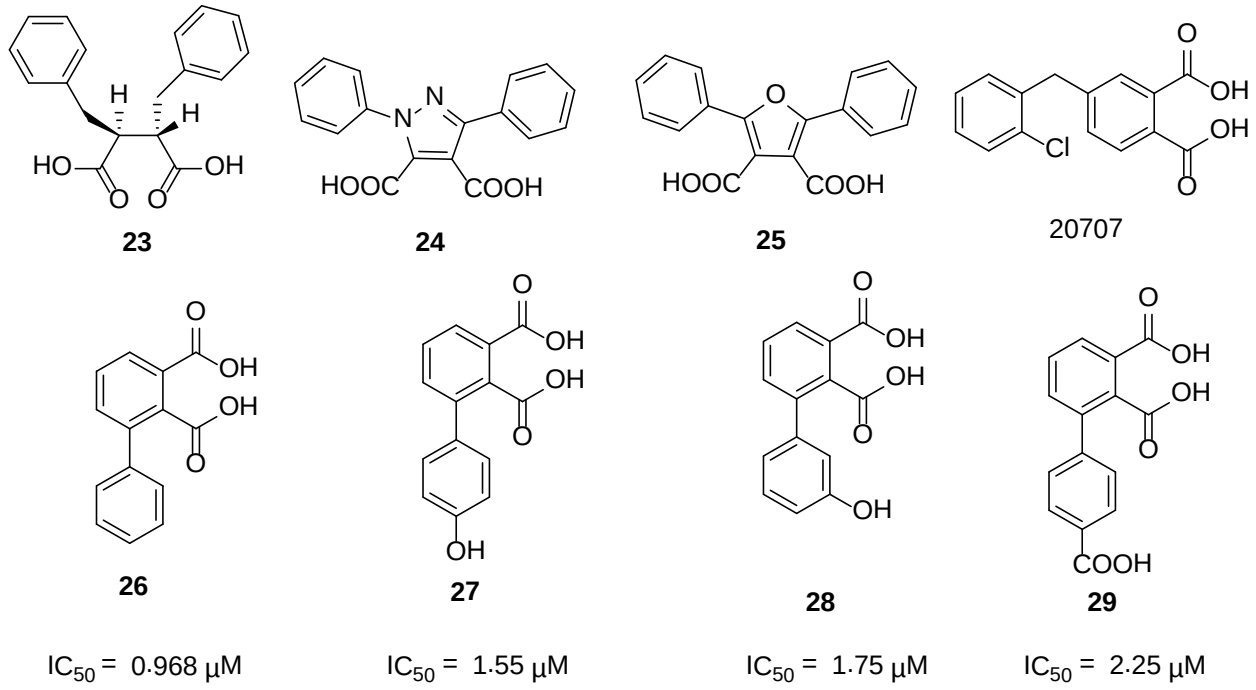
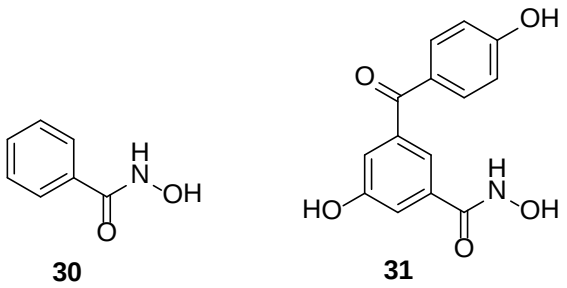


Fig. 6. Succinic and phthalic acid derivatives as MBL inhibitors

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.



30 **31**

Fig. 7. Hydroxamates as FEZ-1 inhibitor

β-Lactam analogues

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*, L1from *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 BCII MBLs with a K_i range 0.10–32.1 μM. Tsang *et al*., [37] reported 8-thioxocephalosporins as weak competitive inhibitors (K_i ~ 700 μM) of *B.cereus* MBL. Interestingly, the hydrolysis product of thioxocephalosporin, 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 sulfones as inhibitors of BIa2 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.

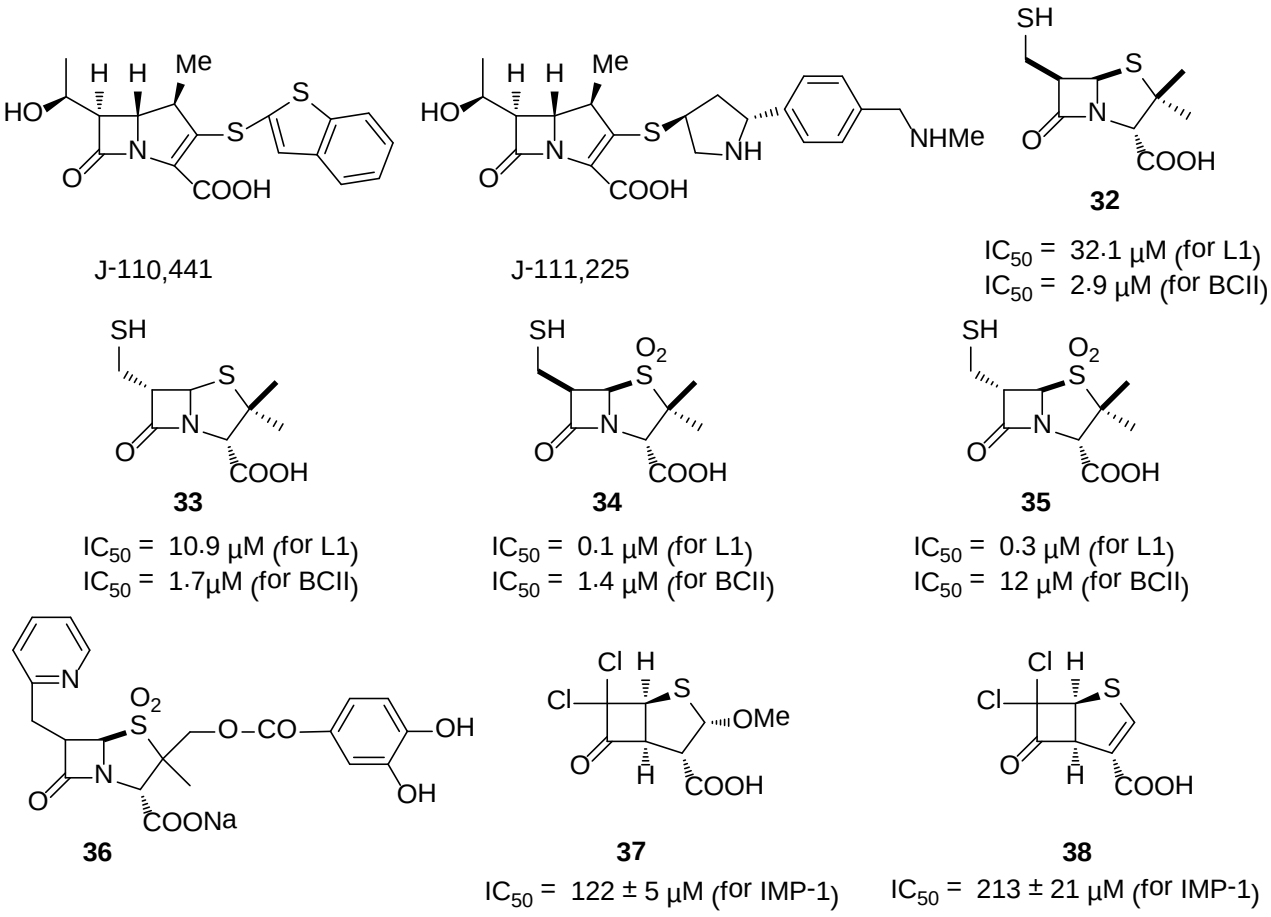


Fig. 8. β-Lactam-derived MBL inhibitors

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 monothiocaraboxylic 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.*

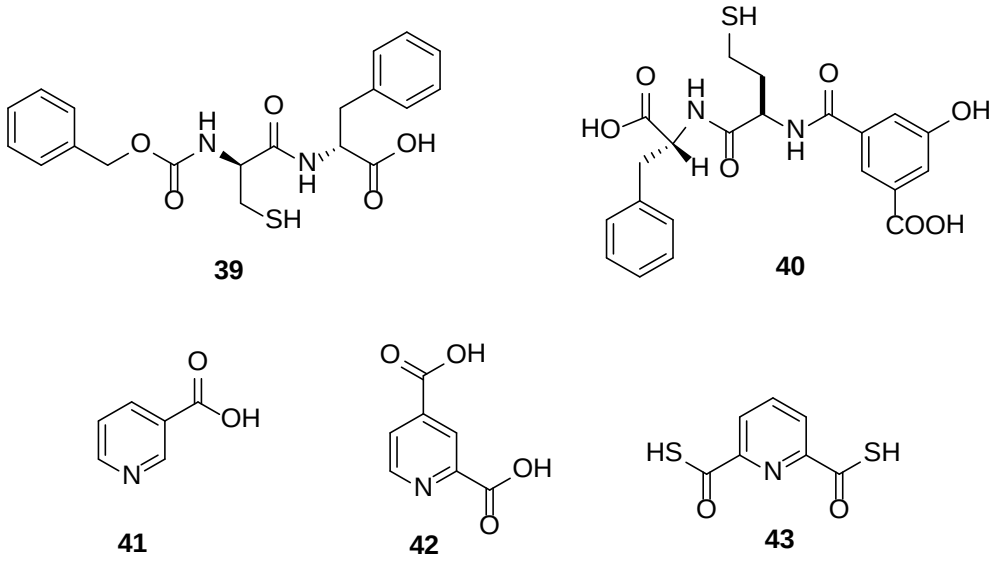


Fig. 9. Cysteinyl peptides and pyridine dicarboxylic acid inhibitors of MBLs

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*.

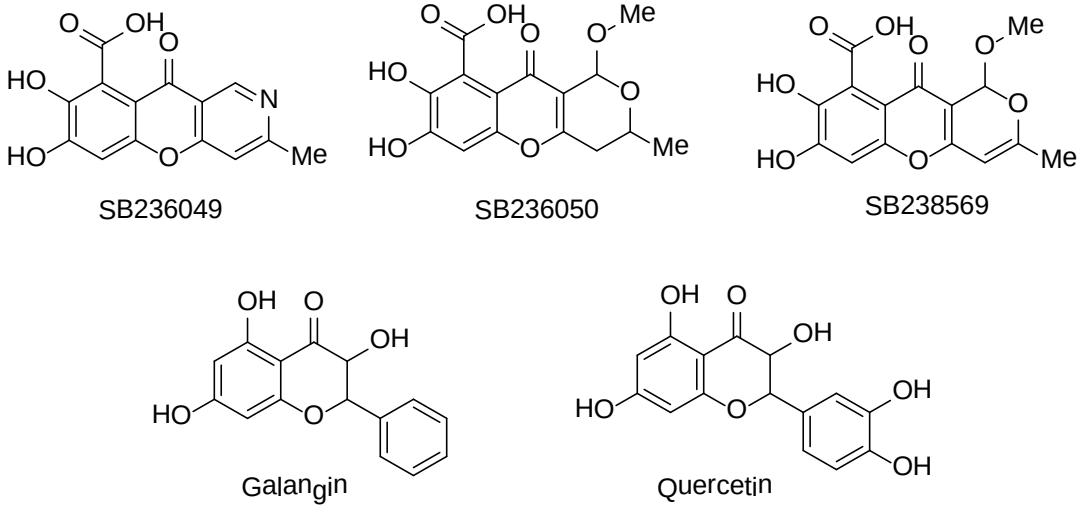


Fig. 10. Natural product-based inhibitors of MBLs

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 ± 0.03 μM) and **45** (K_i = 1.4 ± 0.10 μM), 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)-4*H*-1,2,4-triazole-3-thiol **50** (K_i = 0.97 ± 0.60 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.

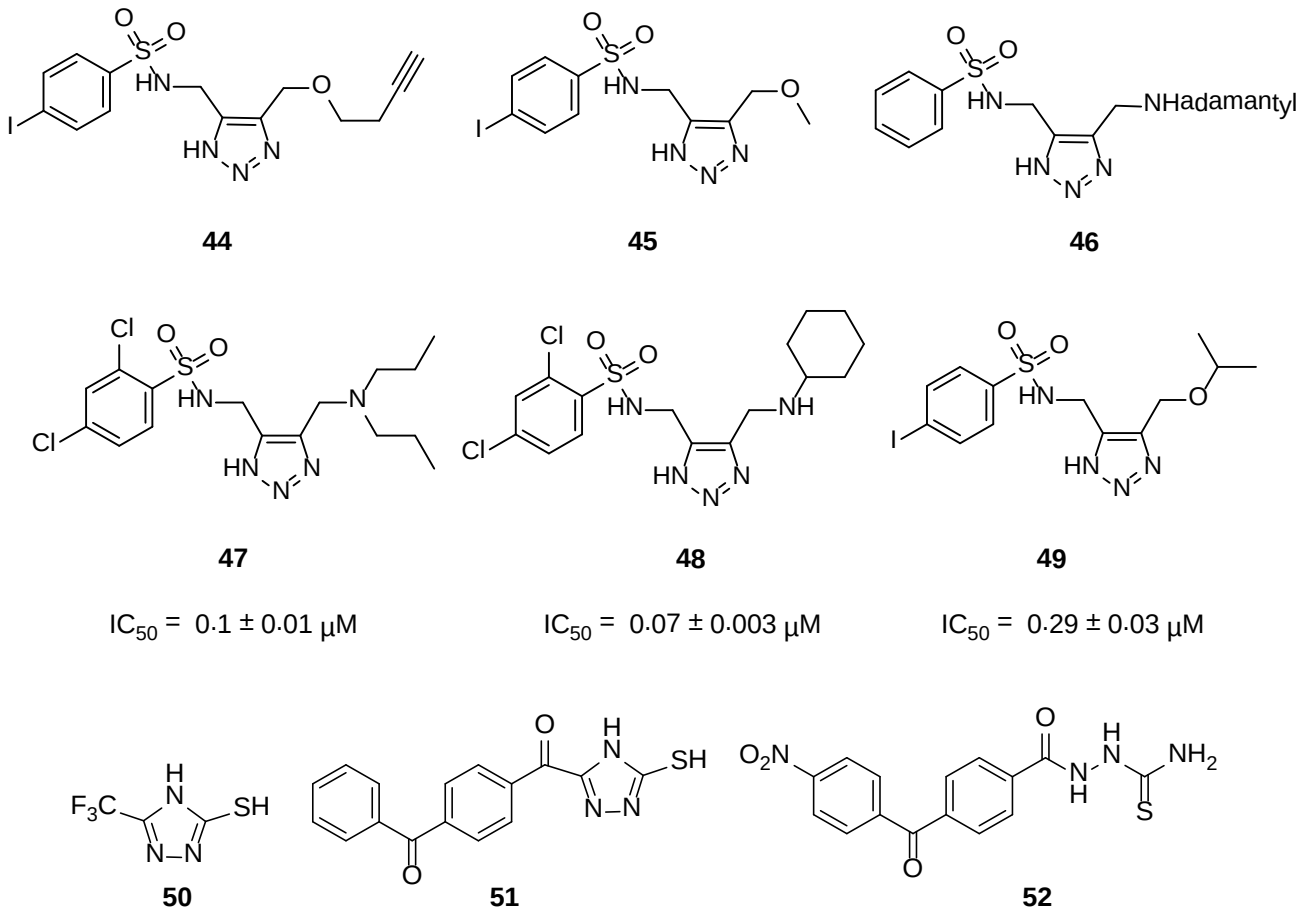


Fig. 11. Triazoles and *N*-acylated thiosemicarbazide inhibitors of VIM-2 and IMP-1 MBLs

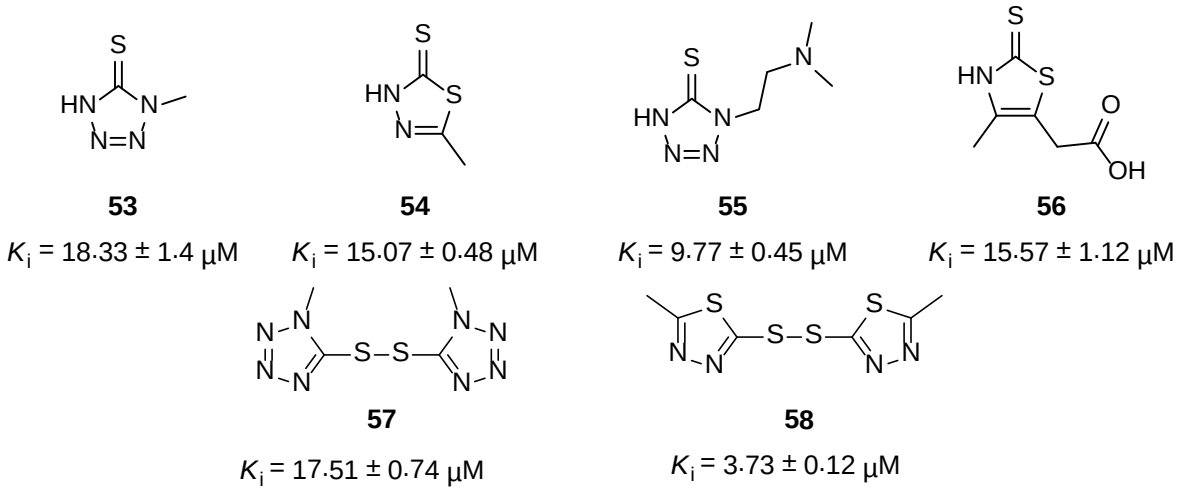


Fig. 12. Thiones and disulfides as BcII enzyme inhibitors

Hetrocyclic Thiones and Disulfides

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 hetrocyclic thiones and disulfides **53–58** (Figure 12) and found that the catalytic activity of the BcII 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 ± 4 μM, while compounds **60** and **61** (Figure 13) have K_i values of 15 ± 5 μM and 18 ± 9 μM, respectively.

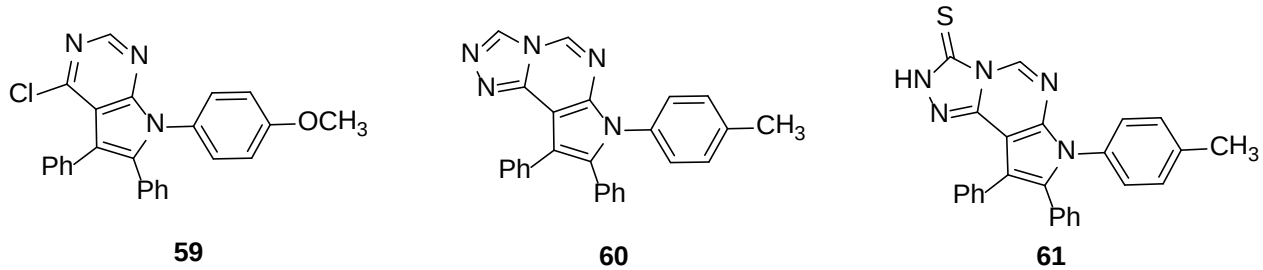


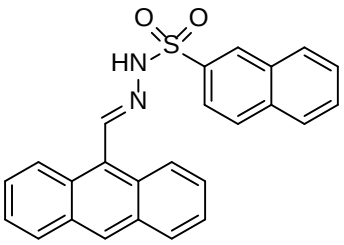
Fig. 13. Pyrrole Based inhibitors of the IMP-1 enzyme

Single Stranded DNAs

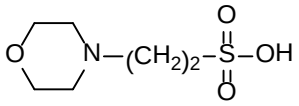
Kim [57] and his colleagues identified single-stranded DNAs as rapid, reversible, and non-competitive inhibitors of *Bacillus cereus* 5 ⁄ B ⁄ 6 MBL, with K_i and K_i' values in the nanomolar range. They assayed these ssDNA's against other zinc-dependent metallo-enzymes like porcine carboxypeptidase A, 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

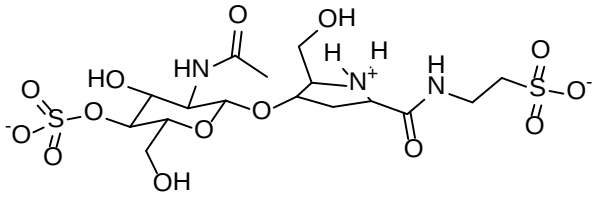
Siemann [58] and his coworkers reported N-arylsulfonyl hydrazones as novel inhibitors of the IMP-1 enzyme. The most potent inhibitor of IMP-1 MBL is compound **62** with an IC_{50} of 1.6 µM. 4-Morpholinoethanesulfonic acid **63** has also been reported as a competitive inhibitor of MBL from *B. fragilis* 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).



62



63



64

Fig. 14. Sulfonated inhibitors of MBLs

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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