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## An updated patent review of metallo- $\beta$ -lactamase inhibitors (2020-2023)

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### Abstract

**Introduction:** Metallo- $\beta$ -lactamases (MBLs) are enzymes produced by bacteria that confer resistance to most  $\beta$ -lactam antibiotics, including carbapenems, which have the broadest spectrum of activity. This resistance mechanism poses a significant threat to public health as it drastically reduces treatment options for severe bacterial infections. Developing effective inhibitors against MBLs is crucial to restore susceptibility to  $\beta$ -lactam antibiotics.

**Areas covered:** This review aims to provide an updated analysis of patents describing novel MBL inhibitors and their potential therapeutic applications that were filed between January 2020 and May 2023.

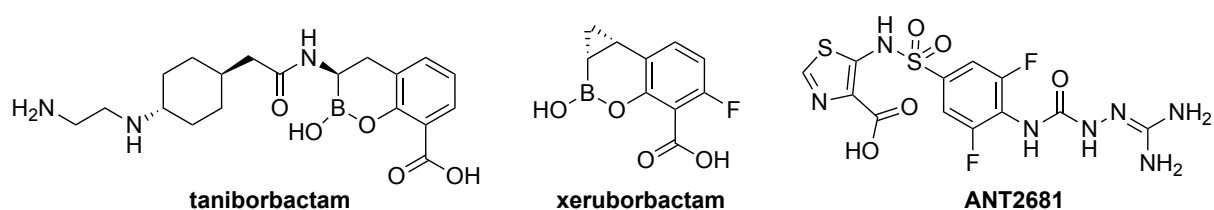
**Expert opinion:** Significant advancements were made in the development of selective MBL inhibitors with zinc-binding and zinc-chelating mechanisms of action. Dual inhibitors, targeting simultaneously both serine- $\beta$ -lactamases (SBLs) and MBLs, represent an interesting alternative approach that is increasingly pertinent for the treatment of infections involving multiple  $\beta$ -lactamases from different Ambler classes. Most examples of MBL-specific inhibitors were focused on the treatment of MBL-mediated infections in *Enterobacterales*, where IMP-1 was a more difficult target compared with VIM-1 or NDM-1, and much less on *Pseudomonas aeruginosa* or *Acinetobacter baumannii*, which are more challenging to address.

## Keywords

Class B, metallo- $\beta$ -lactamases, MBLs, inhibitors, patents, antibiotic resistance, carbapenems

## 1. Introduction

The emergence and spread of antibiotic-resistant bacteria pose a significant challenge to global healthcare systems. Metallo- $\beta$ -lactamases (MBLs) are enzymes capable of hydrolyzing, and thus inactivating a broad range of  $\beta$ -lactam antibiotics, including carbapenems. MBLs, unlike other  $\beta$ -lactamases, open the  $\beta$ -lactam ring through a water molecule coordinated by two zinc ions (subclasses B1 and B3) or one zinc ion (subclass B2) that are present in the active site. No covalent intermediate is formed between the enzyme and substrate. The inhibition of MBLs presents a promising strategy to restore the efficacy of  $\beta$ -lactam antibiotics. While several inhibitors have been developed and are clinically available for serine- $\beta$ -lactamases (SBLs), none is yet clinically available for MBLs. More effort is needed in this field of critical importance, as currently only two inhibitors targeting simultaneously MBLs and SBLs (taniborbactam, formerly known as VNRX-5133 [1,2], and xeruborbactam, formerly known as QPX7728 [3,4]) are in clinical trials [5]. ANT2681 [6] is also a promising molecule acting exclusively against MBLs that is currently progressing towards clinical development (Figure 1).



**Figure 1.** Chemical structures of two inhibitors targeting simultaneously MBLs and SBLs that are currently in clinical trials (taniborbactam and xeruborbactam) and of a MBL inhibitor currently progressing towards clinical development (ANT2681).

This review aims to provide an overview of the recent advancements in the development of MBL inhibitors, focusing on patents filed between January 2020 and May 2023. A number of 19 patents or groups of patents related to this topic were identified for the reference period by searching in the SciFinder<sup>n</sup> database (<https://scifinder-n.cas.org/>) with the query “metallo-

beta-lactamase inhibitors”, filtered by document type (patent) and by publication year (2020-2023), and followed by manual curation.

This review is part of a historical series of papers intended to inform the community about the latest developments in the field of antibiotic resistance, analyzed through the prism of the patents filed: development of new  $\beta$ -lactam antibiotics and  $\beta$ -lactamase inhibitors (2007-2010) [7], antibiotic patents issued in China (1992-2011) [8], and MBL inhibitors (2010-2013 [9] and 2018-2019 [10]).

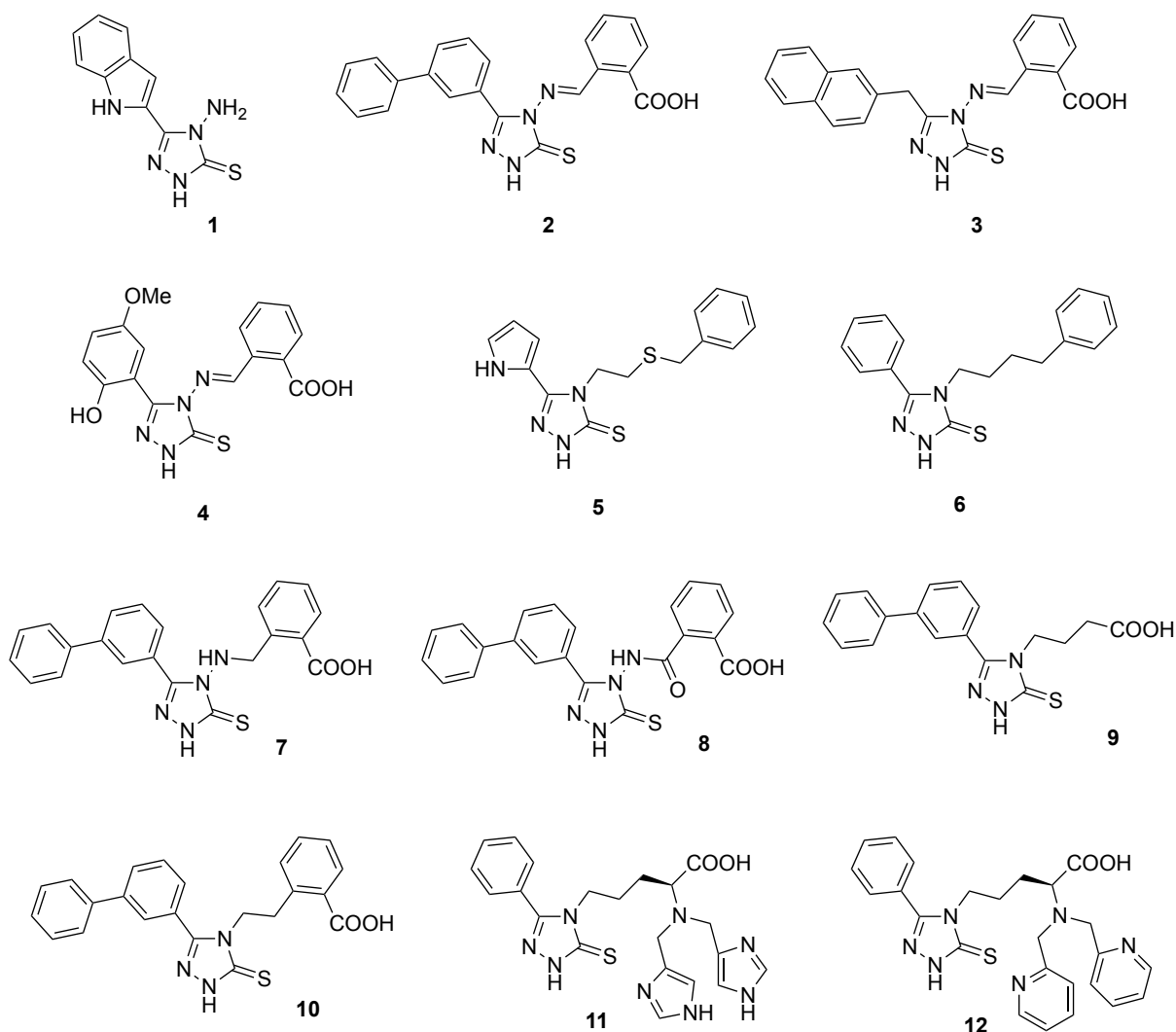
## **2. Metallo- $\beta$ -lactamase inhibitors: Current landscape**

MBL inhibitors function by targeting the active site of the enzyme, either directly or indirectly. Direct inhibitors bind to the zinc ions at the active site, preventing substrate binding and hydrolysis. Indirect inhibitors interfere with the stability or folding of the enzyme, or with its cofactors (the zinc ions in this case), rendering it inactive. In the following sections, we will discuss the MBL inhibitors described during the reference period (January 2020 – May 2023), organized according to their structures and mechanisms of inhibition. No diazabicyclooctane (DBO) inhibitors targeting MBLs were reported during this period.

### 2.1 Zinc-binders

#### *2.1.1. 4-Amino-1,2,4-triazole-3-thione derivatives*

A family of 4-amino-1,2,4-triazole-3-thione derivatives was described in two patents [11,12], and the detailed optimization process for each series was described in several associated papers: unsubstituted amine [13], Schiff base [14], alkylthioether [15], alkylaryl [16], hydrazine [17], hydrazide [17], alkyl [18], 2-ethylbenzoic acid [19], and amino acid [20] series (Figure 2).

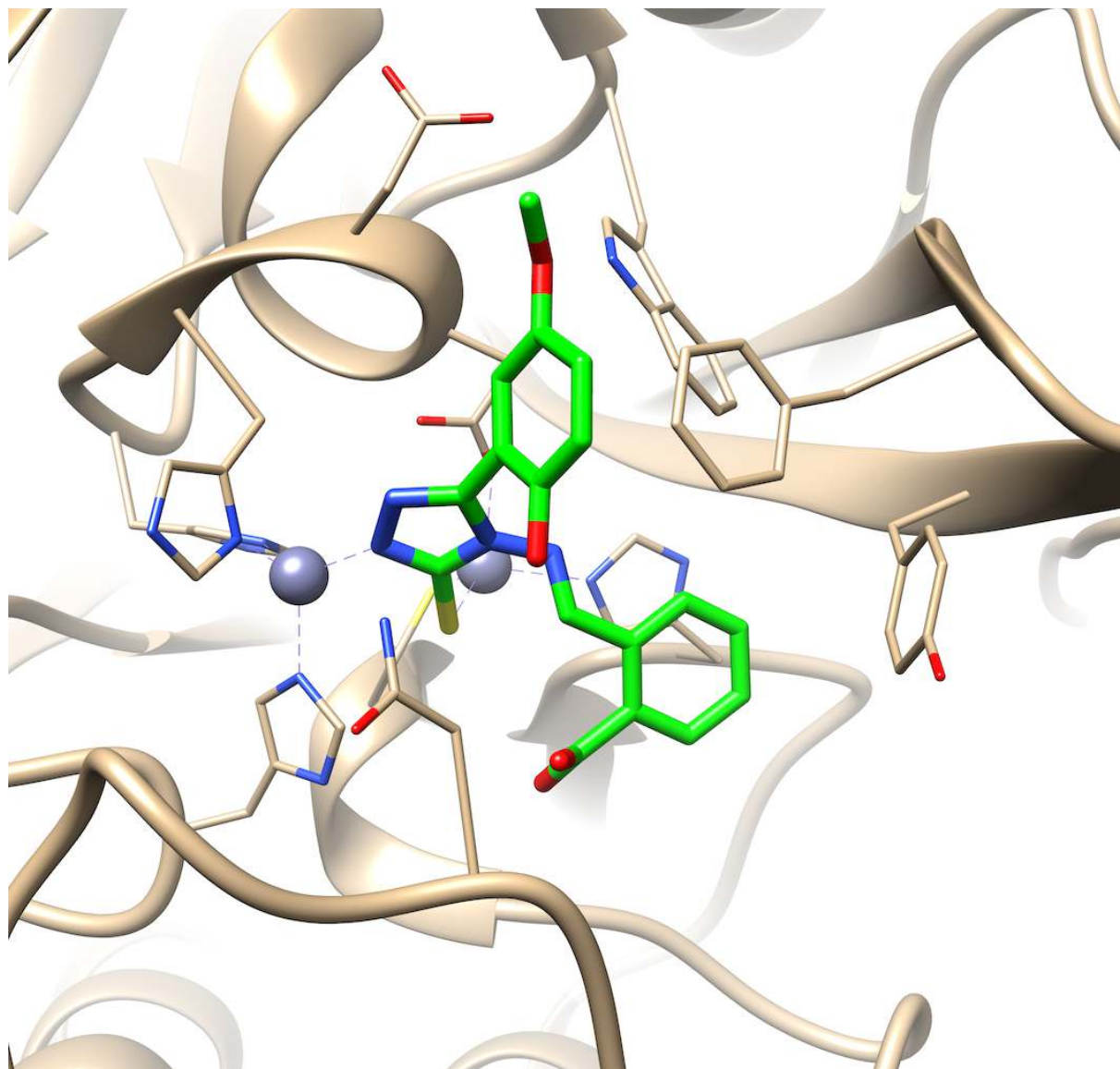


**Figure 2.** Chemical structures of 4-amino-1,2,4-triazole-3-thione compounds (EP3653611 [11], WO2020099645 [12]), representative for the unsubstituted (**1**) [13], Schiff base (**2**, **3** and **4**) [14], alkylthioether (**5**) [15], alkylaryl (**6**) [16], hydrazine (**7**) [17], hydrazide (**8**) [17], alkyl (**9**) [18], 2-ethylbenzoic acid (**10**) [19] and amino acid (**11** and **12**) [20] series.

The initial series, with unsubstituted 4-amino group, included 46 compounds with various substituents in position 5 of the heterocyclic ring [13]. These compounds were prepared in one step from the corresponding carboxylic acids and thiocarbazine (for the aliphatic substituents, Bayer's method) or in three steps from the corresponding esters, hydrazide, and carbon disulfide (for the aromatic substituents), then evaluated for inhibitory activity against five representative MBLs (L1, VIM-4, VIM-2, NDM-1, IMP-1). All compounds were active against at least one enzyme, and the most promising inhibitor (**1**, Figure 2) was active against all five MBLs, with  $IC_{50}$  values ranging from 1.4 to 34  $\mu$ M. In addition, a few compounds

showed significant inhibitory activity towards the B2 enzyme CphA, which suggested that the heterocycle-thione derivatives could also target monozinc MBLs. Some selected compounds were evaluated for selectivity against three MBL-fold enzymes: glyoxalase II from *Plasmodium falciparum*, *exo/endoribonucleases* tRNase Z from *Escherichia coli*, and RNase J1 from *Bacillus subtilis*. With a few exceptions, the compounds were inactive against these enzymes at 100  $\mu$ M or 1 mM [13].

For the synthesis of the Schiff base series, the reaction of several 5-substituted-4-amino-1,2,4-triazole-3-thione derivatives with various aldehydes provided 95 compounds, which were tested for inhibition against six representative MBLs (L1, VIM-2, VIM-4, NDM-1, IMP-1, CphA). Among them, two compounds, **2** and **3** (Figure 2), showed a broad spectrum of inhibition for all six MBLs, with IC<sub>50</sub> values of 0.6, 0.04, 0.2, 4.2, 17.7, 4.0  $\mu$ M and 0.5, 5.0, 2.4, 1.7, 34.4, 15.0  $\mu$ M, respectively [14]. Unfortunately, in spite of good inhibition on purified enzymes, all tested compounds from this series were devoid of synergistic activity when tested in clinical isolates in combination with a  $\beta$ -lactam antibiotic, likely because of low efficiency in bacterial cell penetration. A few selected compounds were also evaluated for selectivity against human glyoxalase II, a bi-zinc metallo-hydrolase belonging to the MBL superfamily, and for cytotoxicity. Two compounds with broad-spectrum MBL inhibition presented clear selectivity in favor of VIM-2, VIM-4, NDM-1, and L1 compared with the human glyoxalase II, and no toxicity was observed at concentrations up to 100  $\mu$ M. In this series of Schiff base derivatives, the thione group was shown to be essential for MBL inhibition [14]. This was further confirmed by the crystal structure (PDB code 6YRP, Figure 3) of VIM-2 in complex with compound **4** (Figure 2), one of the most potent VIM-2 inhibitors identified in this study. The structure showed that the triazole-thione moiety established a simultaneous double coordination of the active site zinc ions, displacing the catalytic hydroxide ion, and the nitrogen at position 2 coordinating Zn1, while Zn2 was interacting with the inhibitor's sulfur atom. A complete electronic density for the Schiff base **4** was clearly present in the VIM-2 active site, showing the absence of hydrolysis of the hydrazone-like bond. Compound **4** interacted with several conserved residues in the active site, including the catalytically important Asn233, His263 and the hydrophobic residues Phe61, Tyr67, and Trp87, located in loop L1 and defining one side of the active cavity [14].

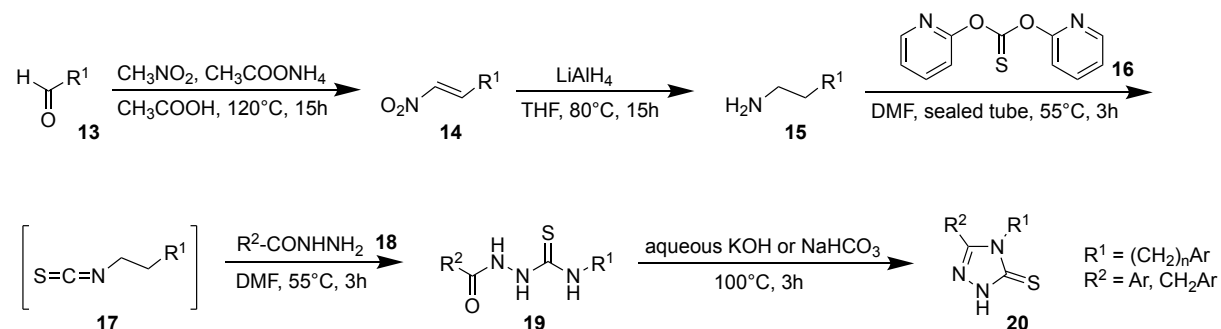


**Figure 3.** Crystallographic structure of the complex between VIM-2 and the Schiff base derivative **4** colored in green (PDB code 6YRP). The ligand and representative active site residues are shown as sticks, and the zinc ions are shown as gray spheres [14].

The synthesis of the alkylthioether derivatives (e.g. compound **5**, Figure 2) was carried out in two steps from aryl hydrazones and aliphatic aminothioethers to provide 30 compounds [15]. They were evaluated as inhibitors of five representative MBLs (VIM-1, VIM-2, VIM-4, NDM-1, IMP-1), showing in most cases micromolar or submicromolar activity against at least one enzyme. The compound **5** (Figure 2) was the most interesting from this series, with a broad spectrum of inhibition ( $K_i$  values of 0.23, 0.34, 0.25, 28, and 9.04  $\mu\text{M}$ , respectively). Selected compounds were evaluated for cytotoxicity on mammalian cells and most compounds (including **5**) did not induce cell lysis ( $\text{IC}_{50} > 250 \mu\text{M}$ ), did not inhibit cell viability and

proliferation ( $IC_{50} > 250 \mu\text{M}$ ) and were not toxic towards Peripheral Blood Mononucleated Cells (PBMC) ( $IC_{50} > 500 \mu\text{M}$ ). Some compounds were also tested *in vitro* for antibacterial synergistic activity with meropenem against two *Klebsiella pneumoniae* strains producing either VIM-1 or VIM-4. Overall, the inhibitors showed a dose-dependent effect, with compound **5** being able to restore a meropenem MIC of 2 mg/mL (16-fold potentiation) at 8  $\mu\text{g/mL}$ . Three crystal structures of VIM-2 complexes with compounds from this series (PDB codes 7OVE, 7OVF, 7OVH) were obtained and showed the same binding mode of the triazole-thione moiety in the di-zinc active site of the enzyme, when compared to previously reported structures [15].

The *N*-alkylaryl derivatives of 5-substituted-4-amino-1,2,4-triazole-3-thione (e.g. compound **6**, Figure 2) were prepared by reacting *N*-alkylarylamines **15**, obtained in two steps from the corresponding aromatic aldehydes **13** via the nitroalkene intermediates **14**, with dipyrildithionocarbonate (DPT) **16** to yield the intermediate isothiocyanates **17** (Scheme 1). These were subsequently treated in the same pot with various hydrazides **18** to form the thiosemicarbazide derivatives **19** and, after cyclodehydration under basic conditions, the expected *N*-alkylaryl derivatives **20** of 5-substituted-4-amino-1,2,4-triazole-3-thione (Scheme 1) [16].



**Scheme 1.** Synthetic route for the preparation of the *N*-alkylaryl derivatives **20** of 5-substituted-4-amino-1,2,4-triazole-3-thione [16].

A number of 62 compounds were prepared according to this procedure, and they were further tested for inhibition against six representative MBLs (VIM-1, VIM-2, VIM-4, NDM-1, IMP-1, L1). Surprisingly, some compounds were inactive against all six enzymes, but others (e.g. **6**, Figure 2) showed a good inhibition of VIM-1, VIM-2, VIM-4 ( $K_i$  values of 1.2, 0.23, and 0.43  $\mu\text{M}$ , respectively), 50-55 % inhibition of NDM-1 and L1 at 100  $\mu\text{M}$ , and no inhibition of IMP-1. Many



compounds presented a good antibacterial synergistic activity with meropenem on VIM-1- and VIM-4-producing *K. pneumoniae* clinical isolates (up to 16-fold potentiation) and only moderate (up to twofold potentiation) on NDM-1-producing *E. coli* clinical isolates. No cytotoxicity was observed for the compounds tested from this series, and the evaluation for selectivity against Angiotensin-Converting Enzyme (ACE, a Zn-dependent peptidase) showed that the inhibition constants of these compounds for ACE are at least 30-fold higher than for VIM-type MBLs [16].

Hydrazine and hydrazide derivatives (e.g. compounds **7** and **8**, Figure 2) were reported as stable analogs of 4-amino-1,2,4-triazole-derived Schiff bases, where the hydrazone-like bond has been reduced (hydrazine series) or the 4-amino group has been acylated (hydrazide series) [17]. Out of 12 compounds from the hydrazine series, only compound **7** (Figure 2) showed significant inhibition of the three MBLs tested (IMP-1, VIM-2, and NDM-1), with  $K_i$  values of 76, 0.24, and 8.2  $\mu\text{M}$ , respectively. Similarly, out of four compounds prepared in the hydrazide series, three were inactive and only compound **8** (Figure 2) was moderately active, with 40 %, 36 %, and 42 % inhibition at 100  $\mu\text{M}$  on the same enzymes. Interestingly, one compound from this series was able to restore the susceptibility of a VIM-2-producing recombinant *E. coli* strain to a cephalosporin (cefoxitin) when tested by disk diffusion at 48  $\mu\text{g}$ . Overall, these results suggest that the main issue would rely on the limited ability of these compounds to penetrate through the bacterial outer membrane and efficiently accumulate in the periplasm [17].

A series of 67 *N*-alkyl-5-substituted-4-amino-1,2,4-triazole-3-thione compounds (e.g. compound **9**, Figure 2) [18] was prepared using the same protocol as described above for the *N*-alkylaryl derivatives (Scheme 1) [16]. These compounds were tested for inhibition against five representative MBLs (VIM-1, VIM-2, VIM-4, NDM-1, IMP-1). Most compounds were inhibitors of the three VIM enzymes with  $K_i$  values in the micromolar and sometimes submicromolar range, only a few of them were able to inhibit NDM-1 ( $K_i$  values in the range 15-100  $\mu\text{M}$ ) and none of them showed a significant inhibition of IMP-1. The most active compound in this series (**9**, Figure 2) was an inhibitor of VIM-1, VIM-2, VIM-4, and NDM-1 with  $K_i$  values of 5.50, 0.35, 0.27, and 99.8  $\mu\text{M}$ , respectively, and devoid of any activity against IMP-1. Microbiological assays showed an antibacterial synergistic activity of selected compounds with meropenem on multidrug-resistant (MDR) VIM-1- and VIM-4-producing *K. pneumoniae* clinical isolates (up to 16-fold potentiation).

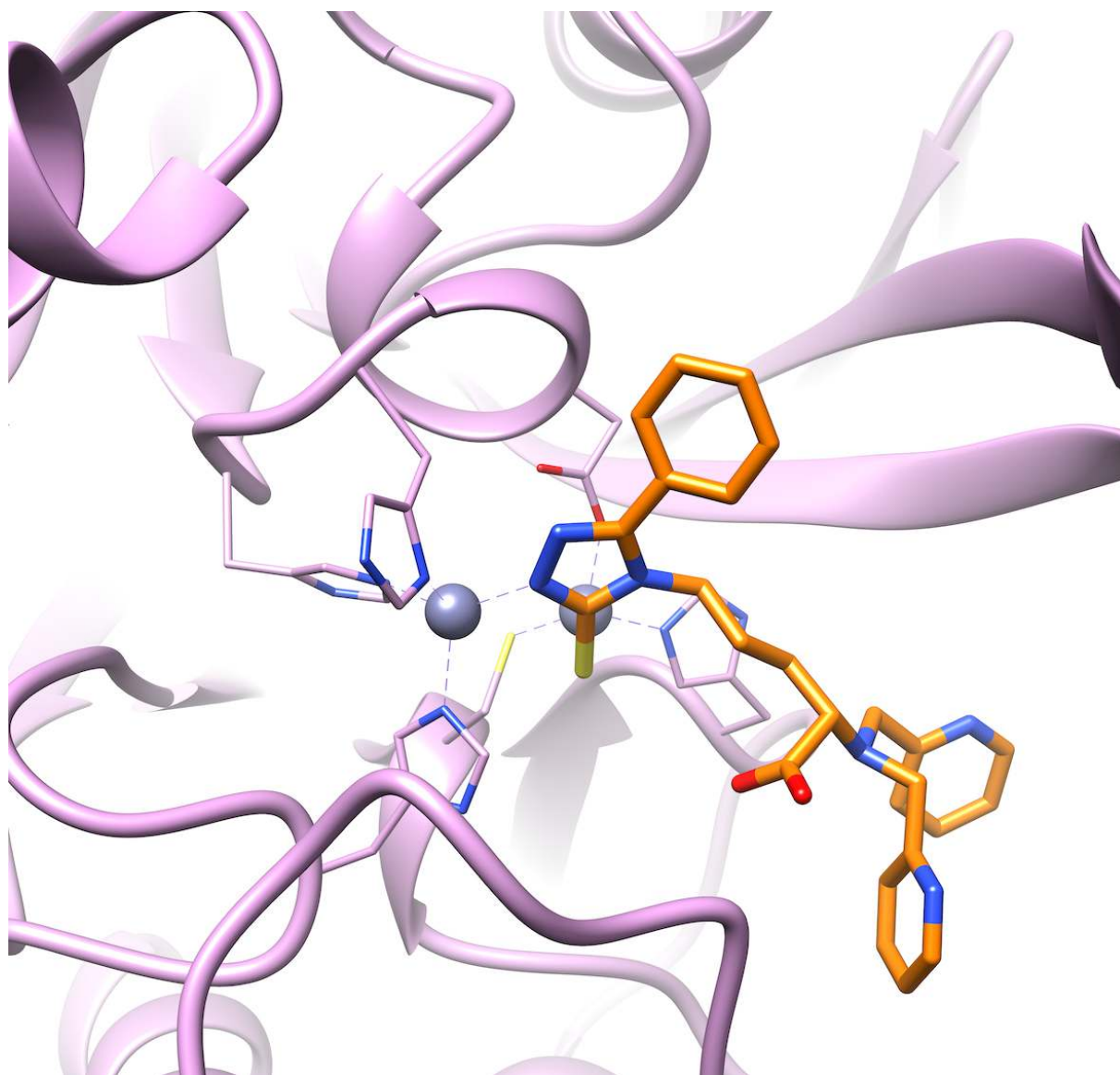
Another series of stable analogues of Schiff bases derived from 5-substituted-4-amino-1,2,4-triazole-3-thiones (e.g. compound **10**, Figure 2) was reported, where the N=C fragment in compound **2** (Figure 2) was replaced by an ethylene CH<sub>2</sub>CH<sub>2</sub> fragment in compound **10** (Figure 2) [19]. The rationale behind this design was to combine the presence of an aromatic group on this substituent, which was associated with a broader spectrum of inhibition, with the presence of an alkyl link, which was found to be significantly beneficial for the antibacterial synergistic activity of molecules. A number of 50 compounds from this series were prepared using the same procedure as for *N*-alkyl [18] and *N*-alkylaryl [16] derivatives, then tested on a panel of five representative MBLs (VIM-1, VIM-2, VIM-4, NDM-1, IMP-1). The majority of compounds were able to inhibit the three VIM enzymes, with K<sub>i</sub> values in the micromolar and submicromolar range, and only very few were slightly active against NDM-1 and IMP-1. The most active compound (**10**, Figure 2) showed K<sub>i</sub> values of 1.61, 0.08, and 2.10 μM against VIM-1, VIM-2, and VIM-4, respectively, and was not active against NDM-1 and IMP-1. Some molecules were able to significantly potentiate the activity of ceftiofuran against a VIM-2-producing *E. coli* strain, restoring the susceptibility to the antibiotic, and to show antibacterial synergistic activity with meropenem on VIM-1- and VIM-4-producing *K. pneumoniae* clinical isolates (up to 16-fold potentiation). The crystal structure of a compound from this series in complex with VIM-2 (PDB code 7PP0) was also reported, showing a binding mode in good agreement with the previous structural studies and structure-activity relationship (SAR) results [19].

The most advanced series in this family is represented by the amino acid derivatives of 4-amino-1,2,4-triazole-3-thiones (e.g. compounds **11** and **12**, Figure 2) [20]. A number of 45 compounds from this series were prepared from the corresponding α-Boc-protected diamine compounds following the same procedure as for *N*-alkyl [18] and *N*-alkylaryl [16] derivatives, then tested as inhibitors on a panel of five representative MBLs (VIM-1, VIM-2, VIM-4, NDM-1, IMP-1). Two compounds, **11** and **12** (Figure 2), were found to be the most promising, with K<sub>i</sub> values of 0.15, 0.41, 0.20, 0.03 μM and 1.7, 0.96, 0.76, 0.57 μM against VIM-1, VIM-2, VIM-4, and NDM-1, respectively. On IMP-1, **12** was inactive and **11** showed 40 % inhibition at 100 μM. However, as the diimidazolyl **11** and dipyridyl **12** derivatives are analogues of compounds known to inactivate NDM-1 by sequestering the active site zinc ions, some potent NDM-1 inhibitors from this series were suspected to inhibit NDM-1 by stripping its zinc cations, and further studies were conducted to better characterize the type of MBL inhibition using

equilibrium dialysis and native state electrospray ionization mass spectrometry (native MS). These studies showed that compounds **11** and **12** are able to diminish the zinc content of both VIM-2 and NDM-1, a phenomenon observed only after a significant period of incubation of the enzyme in the presence of the inhibitor, and suggest that these compounds at least partially inhibited one or both MBLs by zinc stripping. It was shown that compound **11** only partially removed the zinc ions from VIM-2, but it inhibited NDM-1 exclusively by zinc stripping, whereas compound **12** showed a completely opposite behavior, as it fully removed the zinc ions from VIM-2, and showed a mixed inhibition mode against NDM-1. The lower ability of **12** to remove the zinc ions from NDM-1 compared to **11** may explain the 20-fold lower inhibitory potency measured for **12** compared with **11** ( $K_i$  values of 0.57 and 0.03  $\mu\text{M}$ , respectively). By testing the potential synergistic activity of several compounds from this series on MDR MBL-producing clinical isolate at a fixed concentration of 32  $\mu\text{g/mL}$ , many compounds were able to reduce the minimum inhibitory concentration (MIC) of meropenem towards VIM-1- or VIM-4-producing *K. pneumoniae*, VIM-2-producing *P. aeruginosa*, and NDM-1-producing *E. coli* clinical isolates, although the results were not totally correlated with the inhibitory potencies against the corresponding MBLs. The crystal structures of VIM-2 in complex with **11** (PDB code 8A4M, Figure 4) and of NDM-1 in complex with **12** (PDB code 8A76, Figure 5) were obtained with resolutions of 1.98 and 1.50 Å, respectively. The ligand-binding modes and the protein-ligand interactions observed in these structures were in agreement with what was expected from the rational design of these derivatives [20].



**Figure 4.** Crystallographic structure of the complex between VIM-2 and the amino acid derivative **11** colored in magenta (PDB code 8A4M). The ligand and representative active site residues are shown as sticks, and the zinc ions are shown as gray spheres [20].

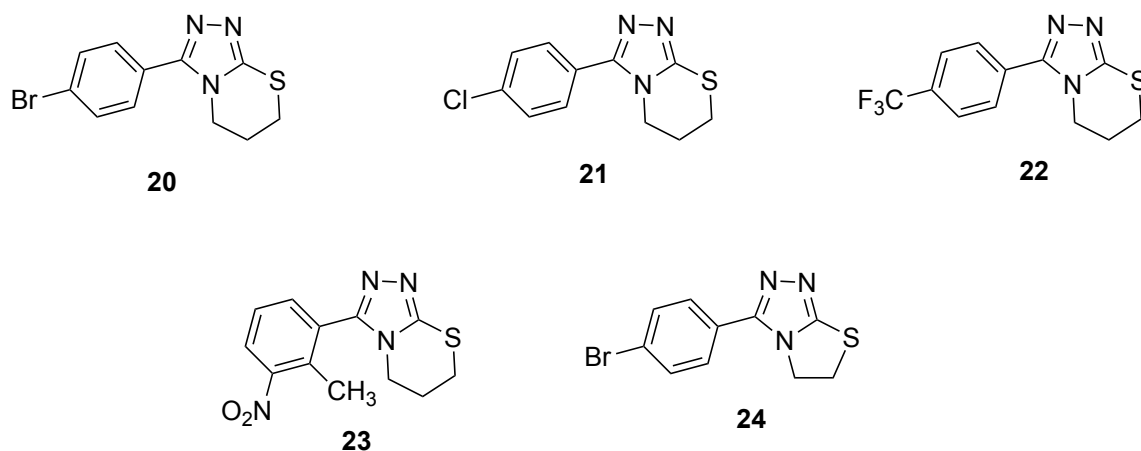


**Figure 5.** Crystallographic structure of the complex between NDM-1 and the amino acid derivative **12** colored in orange (PDB code 8A76). The ligand and representative active site residues are shown as sticks, and the zinc ions are shown as gray spheres [20].

#### 2.1.2. 3-phenyl-6,7-dihydro-[1,2,4]triazolo[3,4-b][1,3]thiazine and 3-phenyl-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3]thiazole compounds

Two series of 3-aryl-6,7-dihydro-[1,2,4]triazolo[3,4-b][1,3]thiazines and 3-phenyl-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3]thiazoles (25 compounds overall) were synthesized in three steps starting from benzoic anhydride and thiosemicarbazide [21]. The MBL inhibitory activity of these compounds was tested at concentrations of 100  $\mu\text{M}$  and 10  $\mu\text{M}$  against purified NDM-1, IMP-1, VIM-1, VIM-2, and VIM-5 enzymes.  $\text{IC}_{50}$  values were then measured against VIM-2 and IMP-1 for the most promising 11 compounds. Among them, five compounds (**20-24**,

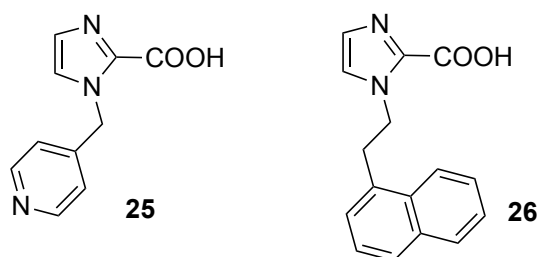
Figure 6) showed  $IC_{50}$  values lower than 100  $\mu M$  against VIM-2 (38.26, 47.24, 53.20, 53.85, and 67.16  $\mu M$ , respectively) [21].



**Figure 6.** Chemical structures of 3-phenyl-6,7-dihydro-[1,2,4]triazolo[3,4-*b*][1,3]thiazine (**20-23**) and 3-phenyl-5,6-dihydro-[1,2,4]triazolo[3,4-*b*][1,3]thiazole (**24**) compounds from CN110840897 [21]

### 2.1.3. 1-substituted-1*H*-imidazole-2-carboxylic acid compounds

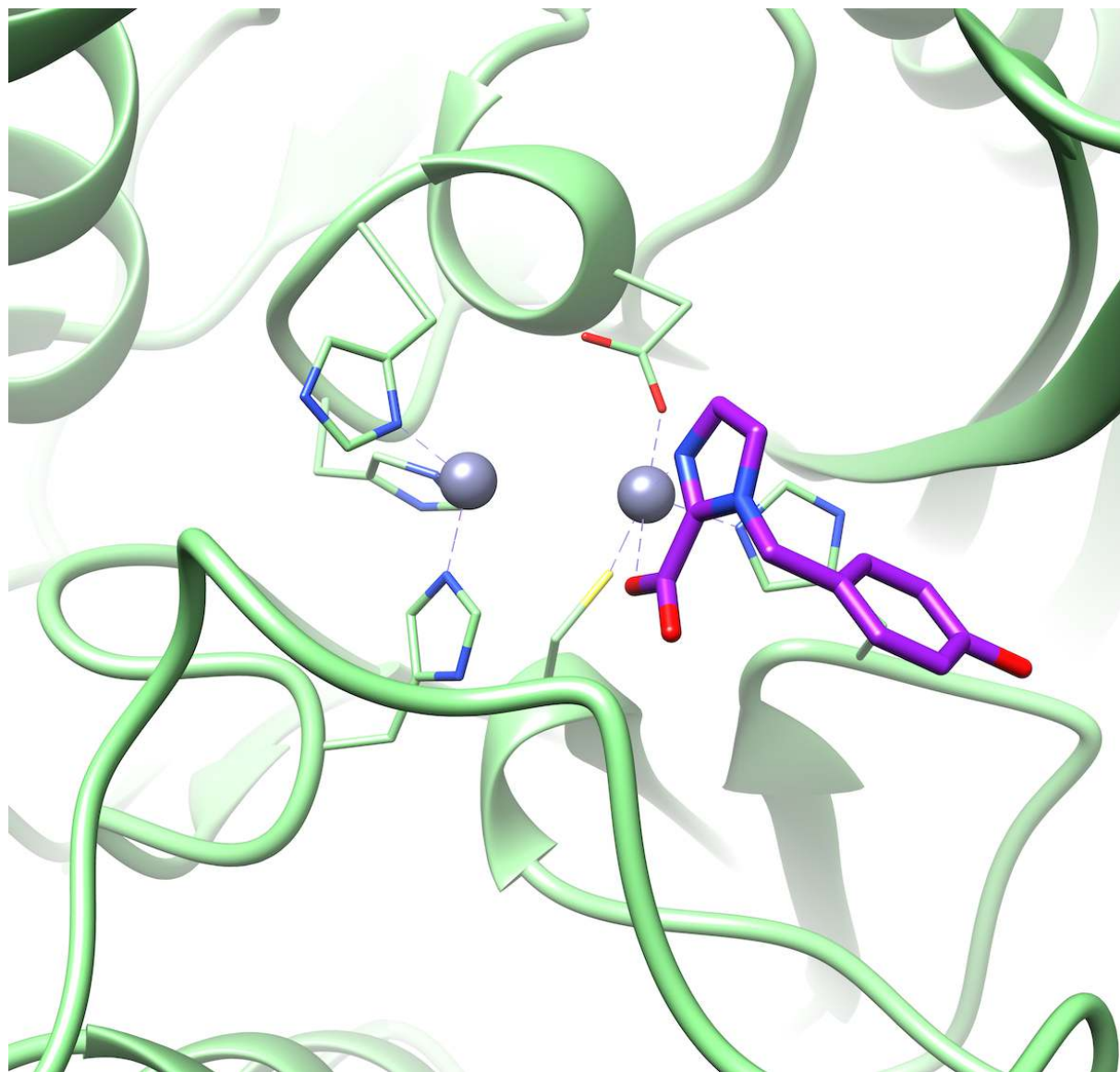
A series of 1-substituted-1*H*-imidazole-2-carboxylic acid derivatives was reported in a patent [22] and in two associated papers [23,24]. These compounds were prepared in two steps by alkylation of the ethyl 1*H*-imidazole-2-carboxylate with the corresponding alkyl or benzyl halide, followed by ester hydrolysis. A few compounds containing different heterocyclic systems (thiazole, oxazole, triazole, benzimidazole) were also synthesized. All compounds were evaluated for MBL inhibition against a panel of five representative MBLs (VIM-2, VIM-1, VIM-5, NDM-1, IMP-1), showing that most compounds are able to inhibit all three VIM enzymes with  $IC_{50}$  values in the micromolar and submicromolar range, but only a few are active on NDM-1 and IMP-1. Compound **25** (Figure 7) showed an inhibition profile restricted to the VIM enzymes, with  $IC_{50}$  values of 0.56, 29.50, 5.78  $\mu M$  against VIM-2, VIM-1, and VIM-5, respectively, whereas compound **26** (Figure 7) has a broader spectrum of inhibition, covering all five enzymes tested, with  $IC_{50}$  values of 0.07, 4.13, 1.07, 74.76, 50.86  $\mu M$  against VIM-2, VIM-1, VIM-5, NDM-1, and IMP-1, respectively.



**Figure 7.** Chemical structures of 1-substituted-1*H*-imidazole-2-carboxylic acid derivatives **25** [24] and **26** [23] from CN111253317 [22]

As these derivatives contain a metal binding pharmacophore (i.e., imidazole-2-carboxyl acid), their zinc chelation ability in solution was evaluated in more detail. It was shown that these compounds do not have strong zinc chelation ability in solution, since their activity was not significantly affected by zinc ion concentrations in the assay media. Selected compounds from this series were evaluated for synergistic antibacterial activity at 10 µg/mL in combination with meropenem against clinical *E. coli* strains producing VIM-2 or IMP-1. Several compounds showed a strong potentiation efficiency ( $\geq 64$ -fold) against VIM-2 expressing *E. coli* strains, but all were inactive against the *E. coli* strain expressing IMP-1. The mechanism of action of these compounds (including **26**) was further investigated, showing that the morphology of *E. coli* cells was not changed when treated with the MBL inhibitors alone. However, in combination with meropenem, the cells became elongated and filamentous, suggesting that the morphological changes are mediated by PBP3 inhibition [23]. Similarly, compound **25** was able to potentiate at a concentration of 10 µg/mL the meropenem activity by fourfold against VIM-2-producing clinical *P. aeruginosa* isolates. Compound **25** possesses a good water solubility ( $>20$  mg/mL). The pharmacokinetic properties of this compound assessed in rats with a single dose of 100 mg/kg showed that plasma concentration reached a peak about 9 min after injection, with an effective maximum concentration of 142.8 µg/ml. The  $t_{1/2}$  value observed for **25** was similar with those of meropenem (1.24 h and approximately 1 h, respectively). A single-dose toxicity study in mice showed no significant toxic effects at doses up to 2000 mg/kg [24]. This study also reported an important number of crystallographic structures of complexes between VIM-2 and various compounds from this series (PDB codes 7DU1, 7DUB, 7DUE, 7DUX, 7DUY, 7DUZ, 7DV0, 7DV1, 7DYY, 7DYZ, 7DZ0, 7DZ1 – see the Beta-Lactamase DataBase (BLDB, <http://bldb.eu>) [25] for a complete list of relevant structures and Figure 8 for a representative example). These structures revealed the importance of the flexible L3/L10

loops in the interaction with the ligands, and also that C4-substituents have unfavorable effects on the ligand coordination to Zn<sup>2+</sup>, while C5-substituents are positioned in a favorable position to interact with the L3 loop residues in VIM-2. However, the SAR analysis showed that the C5-substituents are unfavorable for IMP-1 [24].

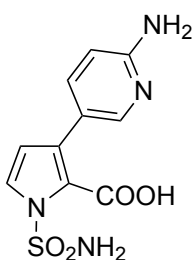


**Figure 8.** Crystallographic structure of the complex between VIM-2 and 1-(4-hydroxybenzyl)-1*H*-imidazole-2-carboxylic acid, colored in purple (PDB code 7DV1). The ligand and representative active site residues are shown as sticks, and the zinc ions are shown as gray spheres [24].

#### 2.1.4. 3-Substituted-1-sulfamoyl-pyrrole-2-carboxylic acid derivatives



A series of 3-substituted-1-sulfamoyl-pyrrole-2-carboxylic acid derivatives acting as MBL inhibitors were described in three patents [26-28] and in an accompanying paper [29]. A number of 95 compounds were synthesized in several steps starting from methyl 3-bromo-1*H*-pyrrole-2-carboxylate and then evaluated as inhibitors of NDM-1. Most compounds showed inhibition in the submicromolar range. One of the most active compounds (**27**, Figure 9) reduced the MIC of meropenem by up to 128-fold in *E. coli* and *K. pneumoniae* producing NDM-1, IMP-1, or VIM-1 MBLs. Supplementation of meropenem with compound **27** resulted in a 32-fold reduction in meropenem MIC for an MDR *K. pneumoniae* strain, indicating that this compound retains the ability to enhance susceptibility to meropenem in highly resistant organisms. Moreover, compound **27** was found to be non-toxic *in vitro* and well tolerated *in vivo*, and demonstrated potent and specific inhibitions of bacterial MBLs compared with human zinc-dependent MMPs [26-29].



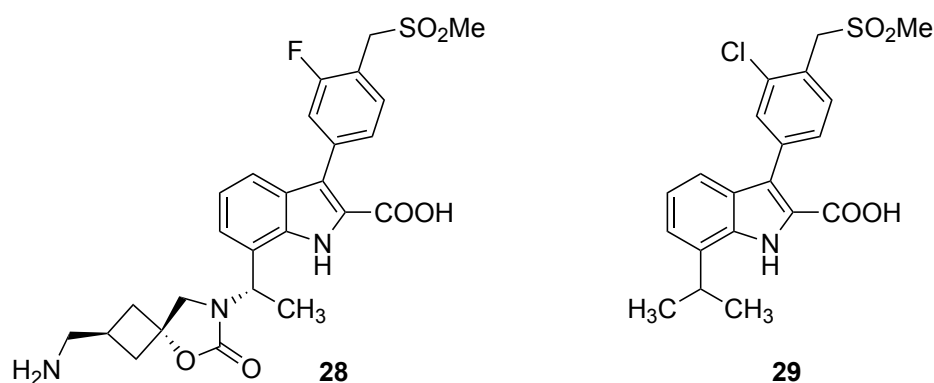
**27**

**Figure 9.** Chemical structure of 3-(6-amino-3-pyridyl)-1-sulfamoyl-pyrrole-2-carboxylic acid **27** [29] from WO2021099793 [26], GB2602096 [27] and WO2022074385 [28]

#### 2.1.5. Indole-2-carboxylic acid derivatives

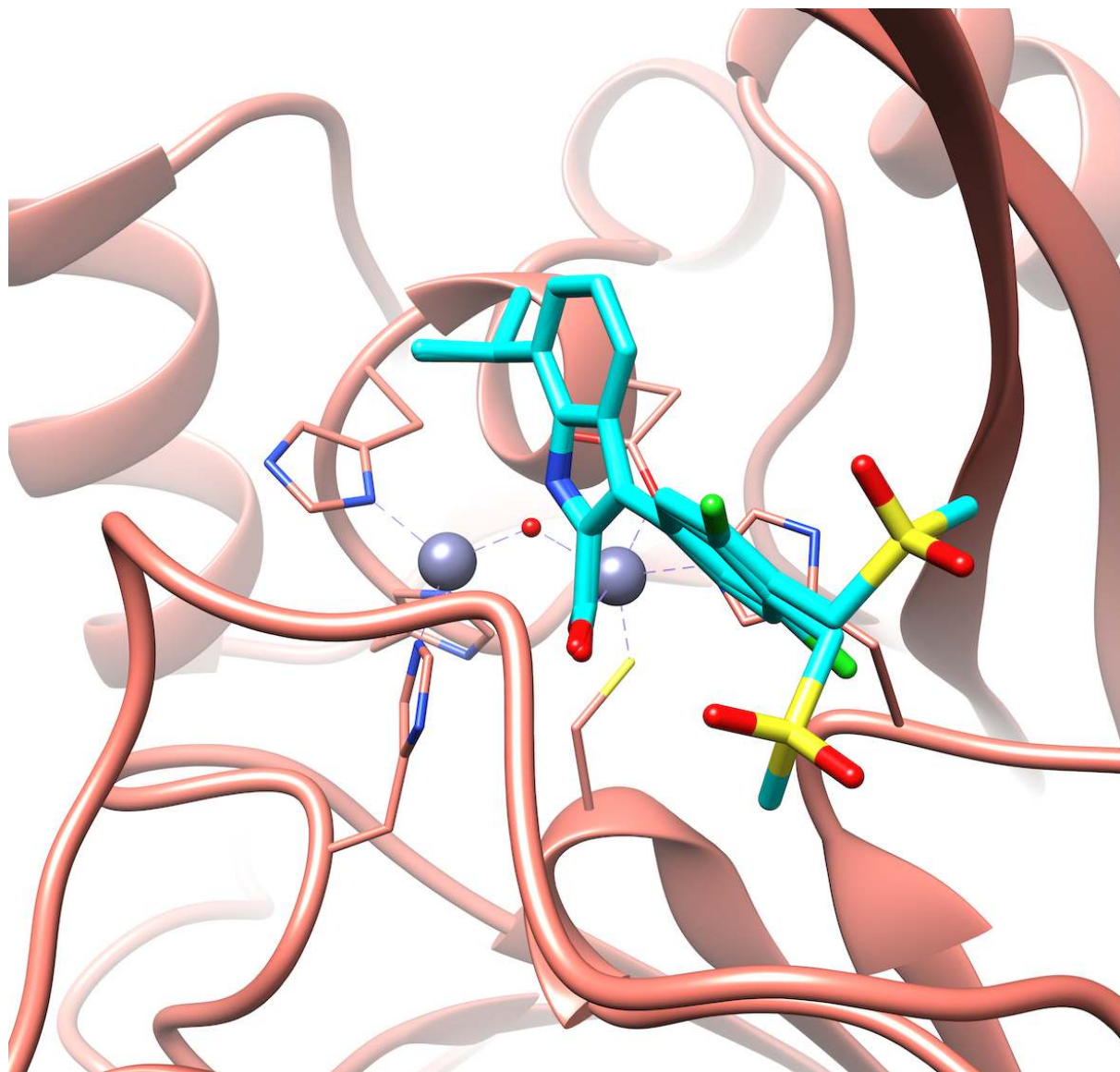
Indole-2-carboxylic acid derivatives were reported as a new class of potent broad-spectrum MBL inhibitors in a patent [30] and in an associated paper [31]. A number of 59 compounds were prepared and tested for inhibition against four representative MBLs (NDM-1, VIM-2, IMP-1, VIM-1). Nanomolar inhibition was observed for some advanced derivatives against all four MBLs tested. Compound **28** (Figure 10) was selected for more advanced studies, showing up to 256-fold potentiation in combination with meropenem on a large panel of Gram-negative bacteria producing NDM-1. A thorough physico-chemical and pharmacologic characterization evidenced that compound **28** was well tolerated and displayed strong

efficacy when combined with meropenem in mouse peritonitis and thigh models with infection by carbapenem-resistant extensively drug-resistant (XDR) strains.



**Figure 10.** Chemical structure of indole-2-carboxylic acid derivatives **28** and **29** [31] from WO2022248887 [30].

In the same study, four crystallographic structures (PDB codes 7AEZ, 7AEX, 7AFY, 7AFZ) of various MBLs (NDM-7, VIM-1, VIM-2, L-1) in complex with indole-2-carboxylic acid derivatives (e.g. compound **29**, Figure 10) were reported, showing that they employ a novel MBL binding mode, imitating the interaction of the antibiotic with MBLs [31,32] (Figure 11). Interestingly, in this binding mode, one of the C7 isopropyl methyl groups of the compound **29** locks the hydroxide in its Zn-Zn bridging location (Figure 11) [30,31].

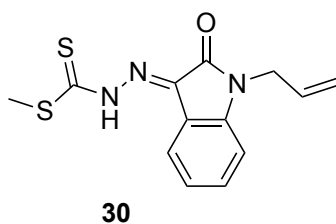


**Figure 11.** Crystallographic structure of the complex between VIM-2 and the indole-2-carboxylic acid derivative **29**, colored in cyan (PDB code 7AFY). The ligand and representative active site residues are shown as sticks, the zinc ions are shown as gray spheres and the hydroxide ion coordinated between them is shown as a small red sphere. The sulfone moiety of the ligand presents two alternate conformations [31].

#### 2.1.6. *N*-allyl-indoline-2-ketone-3-hydrazino-dithioformate derivatives

Several *N*-allyl-indoline-2-ketone-3-hydrazino-dithioformate derivatives were reported as MBL inhibitors [33]. A number of 16 compounds from this series were prepared in two steps from indoline-2,3-diketone, allyl bromide, and carbazyl dithioformate and then tested for inhibition against NDM-1. All compounds showed IC<sub>50</sub> values in the micromolar range, with

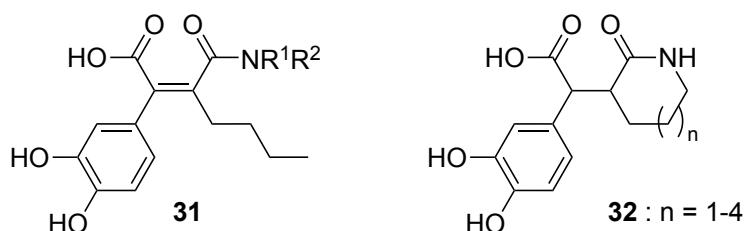
the best inhibition measured for the compound **30** (Figure 12). Some compounds from this series were able to reduce by twofold the MIC of an *E. coli* strain producing NDM-1 [33].



**Figure 12.** Chemical structure of methyl *N*-(2-hydroxy-1-prop-2-enylindol-3-yl)iminocarbamodithioate **30** from CN115974759 [33]

#### 2.1.7. 3,4-Dihydroxyphenylacetic acid derivatives

A series of 3,4-dihydroxyphenylacetic acid derivatives containing alkylidene-amide (**31**) or lactam rings of different sizes (**32**) as substituents in the benzyl position (Figure 13) were prepared and evaluated as MBL inhibitors [34]. Most compounds from this series showed micromolar or submicromolar  $IC_{50}$  against IMP-1 and selected compounds were able to restore the susceptibility to ceftazidime or meropenem against MBL-producing *E. coli*, *K. pneumoniae*, and *P. aeruginosa* strains [34].

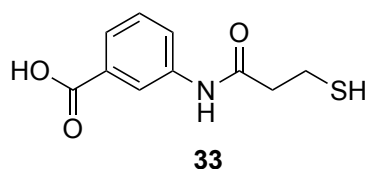


**Figure 13.** Chemical structure of compounds **31** and **32** from JP2022041072 [34].

#### 2.1.8. *N*-Aryl-3-mercaptopropanamides

A series of polar *N*-aryl-3-mercaptopropanamide inhibitors of MBLs was described in a patent [35] and an associated paper [36]. A number of 38 compounds were synthesized in 1-2 steps from the corresponding aniline and 3-mercaptopropionic acid or 3-(acetylthio)mercaptopropionic acid and then tested against three representative subclass B1 MBLs (NDM-1, IMP-7, VIM-1). All compounds were active in the micromolar or submicromolar range. The compound **33** (Figure 14) showed promising activities ( $IC_{50}$  values of 4.0, 6.4, and

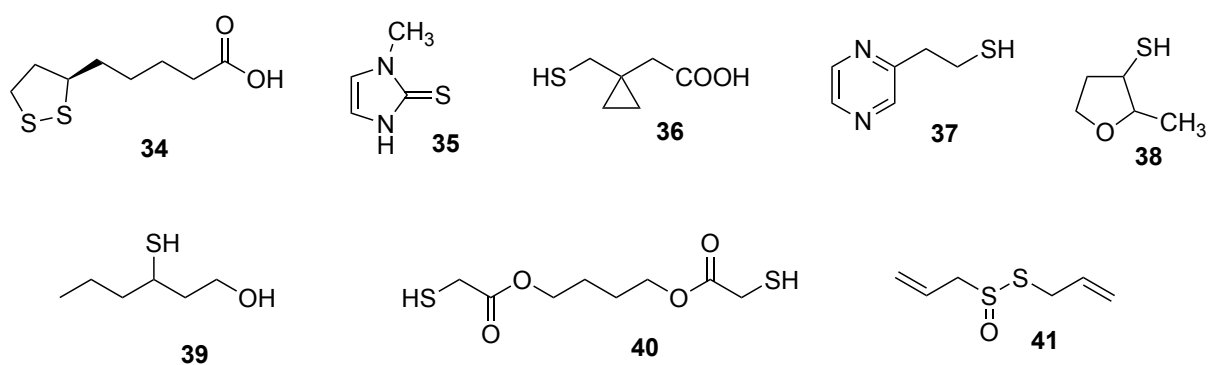
1.2  $\mu\text{M}$  against the three enzymes, respectively) and was selected for more detailed studies. When several compounds were tested for selectivity against six human matrix metalloproteases (MMP-1, -2, -3, -7, -8 and -14) and against the human off-target tumor necrosis factor- $\alpha$ -converting enzyme (TACE), most of them were inactive or showed low level of inhibition at 100  $\mu\text{M}$ . Compound **33** demonstrated a synergistic effect with imipenem against *E. coli* strains producing NDM-1 in a concentration-dependent manner, being able to reduce the MIC values up to 256-fold. Furthermore, the combination of imipenem with **33** showed *in vivo* efficacy in a *Galleria mellonella* model, increasing the survival rate of infected larvae by up to 31%.



**Figure 14.** Chemical structure of 3-(3-mercaptopropanamido)benzoic acid **33** [36] from WO2021191219 [35]

#### 2.1.9. Sulfur-containing small molecules

$\alpha$ -Lipoic acid **34** (Figure 15) is a natural co-factor of several enzymes in various organisms. It shows moderate inhibitory activity against NDM-1, with an  $\text{IC}_{50}$  of 62.75  $\mu\text{M}$ , but is not active against VIM-2 and IMP-7. It was reported to reduce, in combination with meropenem, more than 512-fold the MIC of *E. coli* strains expressing NDM-1, VIM-2, IMP-7 (and also KPC-2), but the mechanism of this *in vivo* activity in the absence of MBL inhibition is not known [37].



**Figure 15.** Chemical structures of  $\alpha$ -lipoic acid **34** from CN112999220 [37], methimazole **35** from CN113209090 [38], 2-(1-mercaptomethyl-cyclopropyl) acetic acid **36** from CN113521044

[39], 2-pyrazinylethylthiol **37**, 2-methyl-3-tetrahydrofuryl mercaptan **38**, 3-thio-1-hexanol **39** and 1,4-butanediol-*bis*(thioglycolate) **40** from CN113425726 [40] and diallyl thiosulfinate **41** from CN114392253 [41].

Methimazole **35** (Figure 15), also known as thiamazole, is an approved drug used for the treatment of hyperthyroidism. It was shown to inhibit NDM-1 with a  $K_i$  of 57.16  $\mu\text{M}$ , but no activity could be evidenced against VIM-2 and NDM-7. In combination with meropenem, compound **35** was able to reduce up to eightfold the MIC of *E. coli* strains expressing NDM-1, VIM-2 or IMP-7. However, the mechanism of this *in vivo* activity in the absence of MBL inhibition is not known [38].

2-(1-Mercaptomethyl-cyclopropyl) acetic acid **36** (Figure 15) was also evaluated as an inhibitor of MBLs, showing moderate activities against the three enzymes tested (NDM-1, VIM-2, IMP-7) with  $K_i$  values of 38.70, 11.21, and 10.96  $\mu\text{M}$ , respectively. Surprisingly, considering the level of inhibition of MBLs, this compound was also able, in combination with meropenem, to reduce with more than 4000-fold the MIC of *E. coli* strains expressing NDM-1, VIM-2, or IMP-7 [39].

In another study [40], several sulfhydryl-containing compounds (2-pyrazinylethylthiol **37**, 2-methyl-3-tetrahydrofuryl mercaptan **38**, 3-thio-1-hexanol **39**, and 1,4-butanediol-*bis*(thioglycolate) **40**, Figure 15) were evaluated as MBL inhibitors against three representative enzymes, NDM-1, VIM-2, and IMP-7. Compounds **37** and **38** were active against all three MBLs, with  $K_i$  values of 39.48, 120.28, 221.34, and 123.98, 198.36, 34.25  $\mu\text{M}$ , respectively. Compound **39** was able to inhibit only IMP-7, with  $K_i$  of 58.54  $\mu\text{M}$ , whereas compound **40** was active only against NDM-1, with  $K_i$  of 16.42  $\mu\text{M}$ . These compounds were reported to reduce, in combination with meropenem, more than 16- to 1024-fold the MIC of *E. coli* strains expressing NDM-1, VIM-2, IMP-7 (and also KPC-2), but the mechanism of this *in vivo* activity in the absence of MBL inhibition is not known [40].

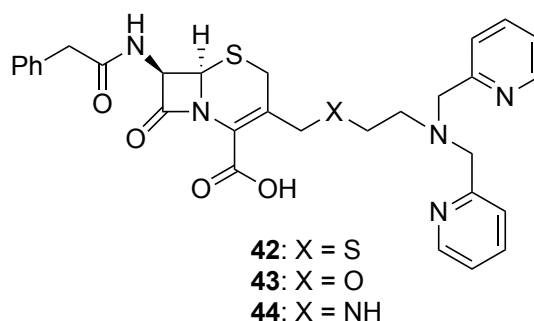
Diallyl thiosulfinate **41** (Figure 15), also known as allicin, is an organosulfur compound that is present in garlic. It was found to have an inhibitory effect on NDM-5, with a  $K_i$  of 17.69  $\mu\text{M}$  [41].

## 2.2. Metal chelator-based inhibitors

This category of inhibitors is able to disrupt the metal ion coordination, which is essential for the MBL activity.

### 2.2.1. $\beta$ -Lactam conjugates with pyridyl-containing chelators

The conjugate molecules **42**, **43**, and **44** (Figure 16) were prepared by coupling 7-phenylacetylaminoccephalosporanic acid with 2,2'-dipicolylamine through an aliphatic linker containing S, O, or NH, respectively [42]. In combination with meropenem, these compounds were able to reduce 32-fold (from 128 to 4) the MIC of *E. coli* strains expressing NDM-1. Moreover, compounds **42-44** showed no cytotoxicity in human cells at concentrations up to 256  $\mu\text{g/mL}$ .

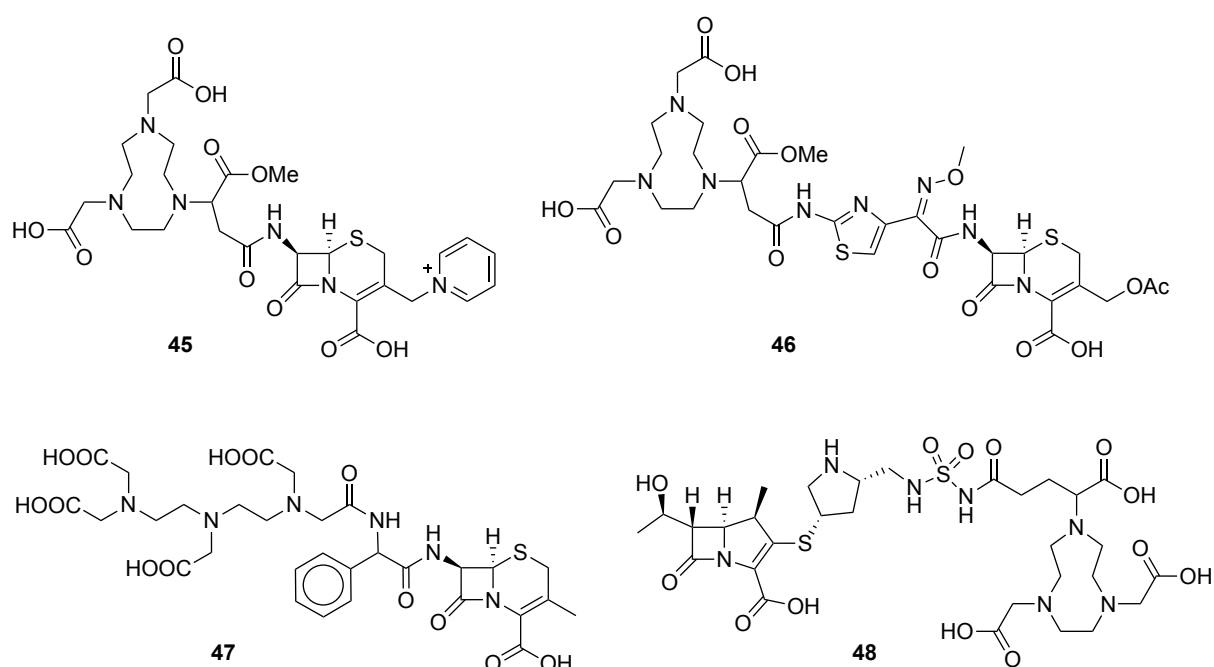


**Figure 16.** Chemical structure of conjugate compounds **42** (X = S), **43** (X = O) and **44** (X = NH) from CN111718361 [42]

### 2.2.2. $\beta$ -Lactam conjugates with carboxylate-containing chelators

A series of 17 compounds containing aza-cycloalkane zinc chelating moieties covalently attached to a  $\beta$ -lactam scaffold (cephalosporin or penicillin) were described in a patent [43] and in two associated papers [44,45]. Based on preliminary evaluations, two compounds from this series (**45** and **46**, Figure 17) were selected for further investigations. Compound **45** (named BP1) possesses a pyridinium substituent in position 3' of a cephalosporin bicyclic ring system and a NOTA chelating group directly attached to the amino group in position 7. In combination with meropenem, it was able to reduce 8- to 32-fold the MIC of *E. coli*, *E. cloacae*, or *K. pneumoniae* strains producing NDM-1, NDM-4, IMP-1, IMP-8, or VIM-1 MBLs. Furthermore, **45** was nontoxic against human hepatocarcinoma cells ( $\text{IC}_{50} > 1000 \text{ mg/L}$ ) and could inhibit the VIM-2 and NDM-1 MBLs with  $K_i$  of 24.8 and 97.4  $\mu\text{M}$ , respectively. No inhibition was observed with the human zinc-containing enzyme glyoxylase II at concentrations up to 500  $\mu\text{M}$ . In mice infected with *K. pneumoniae* producing NDM-1,

compound **45** co-administered with meropenem was able to reduce the bacterial load by  $>3$   $\log_{10}$  units' post-infection [44]. On the other hand, compound **46** (named BP2) contains an acetate substituent in position 3' of a cephalosporin bicyclic ring system and a NOTA chelating group connected to the amino group in position 7 via an oxyimino-aminothiazole linker. This compound was able to inhibit NDM-1 and VIM-2 with  $K_i$  values of 35.3 and 30.9  $\mu\text{M}$ , respectively, and it was not active against glyoxalase II (an MBL-fold zinc enzyme) at concentrations up to 500  $\mu\text{M}$ , indicating a specific MBL binding. According to the antimicrobial susceptibility results, compound **46** potentiates the synergistic activity of meropenem against *Enterobacterales* (*E. coli*, *Enterobacter cloacae*, *K. pneumoniae*, *Serratia marcescens*, *Providencia stuartii*, *P. rettgeri*) producing MBLs (NDM-1, NDM-4, IMP-1, IMP-8, IMP-11, VIM-1, VIM-2, VIM-19) by restoring the meropenem MIC to therapeutically acceptable levels ( $\leq 1$  mg/L). Compound **46** displayed specific activity towards MBLs, as there was no activity exhibited against SBL-expressing variants. In addition, **46** is bactericidal over 24 h and safe to administer at the selected concentrations. In a murine infection model, **46** co-administered with meropenem was efficient, leading to a  $>3$   $\log_{10}$  reduction cfu/thigh in *K. pneumoniae*-producing NDM-1 [45].



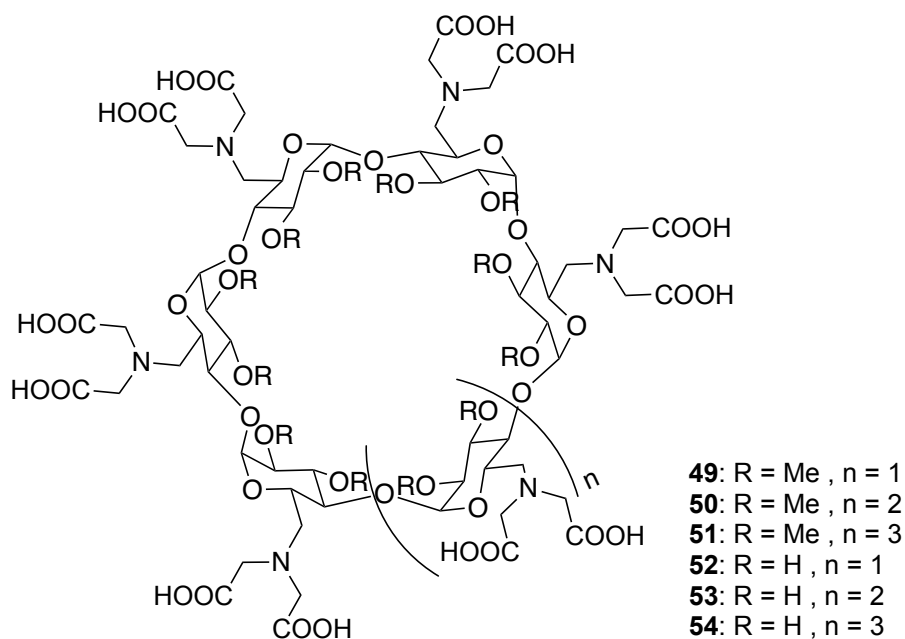
**Figure 17.** Chemical structure of conjugate compounds **45** [44], **46** [45] from WO2023007325 [43], and **47**, **48** from WO2022239872 [46]



Another series of eight conjugate molecules was reported, with cyclic and acyclic metal-chelating moieties attached to penicillin, cephalosporin, and carbapenem scaffolds in positions 6, 7, and 2, respectively [46]. In the acyclic group, these combinations include DTPA bound to cephalexin, cefaclor, cefradine, amoxicillin, and 7-aminodesacetoxycephalosporanic acid, and in the cyclic group the combinations are NOTA-GA bound to cefradine, cephalexin, and doripenem. Several compounds (including **47** and **48**, Figure 17) were able to increase the sensitivity to meropenem (up to 25  $\mu\text{M}$ ) in an *E. coli* strain producing IMP-1. Compound **48** was efficient when tested in combination with meropenem on 16 MDR *P. aeruginosa* strains. Compound **47** was found to be effective (50 % survival rate improvement) when co-administered with meropenem in mice infected with *K. pneumoniae* producing IMP-1.

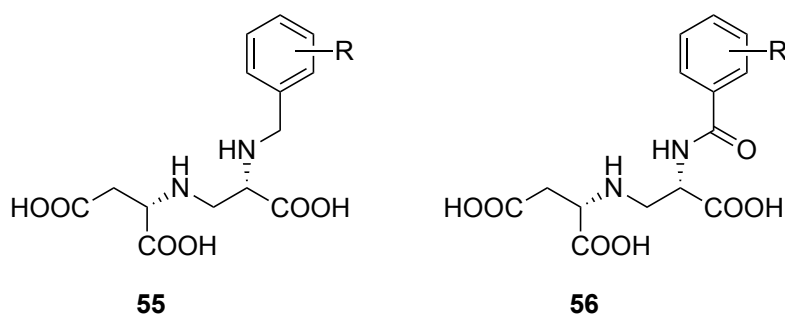
### 2.2.3. Carboxylate-containing chelators

A series of six iminodiacetic acid-substituted cyclodextrins (IDACYDs) (compounds **49-54**, Figure 18) [47] were synthesized and evaluated as MBL inhibitors. First, the *in-vitro* inhibitory activity of these compounds against a *K. pneumoniae* strain producing NDM-1 was demonstrated by NMR spectroscopy. In the absence of inhibitor, after 6-h incubation almost 100 % of meropenem was hydrolyzed, whereas in the presence of the inhibitor (1.9 mM) no signals of hydrolysis of meropenem could be observed over a 12 h period. To verify the direct inhibitory effect on the MBLs, the  $\text{IC}_{50}$  against NDM-1 and VIM-1 were determined spectrophotometrically, with values of 1.63 and 14.99  $\mu\text{M}$ , respectively. When tested using the Kirby-Bauer disc diffusion method against 36 highly resistant Gram-negative bacterial strains producing NDM-, VIM- and IMP-type MBLs, compounds **49-54** were able to reduce meropenem's and imipenem's MIC up to 256-fold, re-sensitizing all MBL-producing strains to carbapenems. No *in-vitro* cytotoxicity was detected at concentrations up to 1,000 mg/L. The acute *in-vivo* toxicity of these compounds was also determined, showing no deaths and no signs indicative of acute toxicity at doses up to 500 mg/kg [47].



**Figure 18.** Chemical structure of compounds **49-54** from EP3714904 [47]

A series of 16 compounds with the generic structures **55** and **56** (Figure 19) were prepared and tested as MBL inhibitors [48]. These compounds showed, in combination with meropenem, a bacteriostatic activity on a drug-resistant *E. cloacae* strain producing NDM-1.

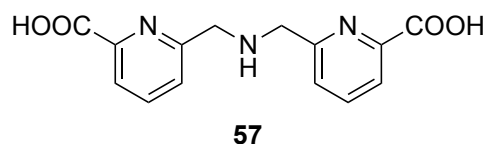


**Figure 19.** Chemical structure of compounds **55** and **56** from CN114085162 [48]

#### 2.2.4. Combined pyridyl- and carboxylate-containing chelators

A series of compounds derived from the structure **57** (Figure 20), which is named H<sub>2</sub>dpa, were synthesized and evaluated as MBL inhibitors [49]. The three derivatives that were prepared possessed *N*-alkylcarboxylic or *N*-alkylphosphonic substituents. These compounds were able to inhibit the NDM-1, IMP-4, and VIM-1 MBLs with IC<sub>50</sub> in the range 1.04-4.84 μM, and to reduce up to 2048-fold, in combination with meropenem, the MIC of an *E. coli* strain producing NDM-1. Toxicity experiments proved that one carboxylic derivative of **57** has low *in-vitro*

cytotoxicity and *in-vivo* toxicity, and *in-vivo* experiments showed that the survival rate of mice infected with MBL-producing *K. pneumoniae* can be remarkably improved by the combined treatment of this derivative and meropenem.

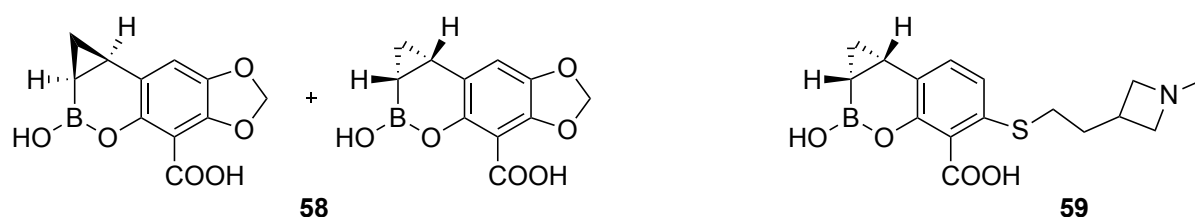


**Figure 20.** Chemical structure of compound **57** from CN113425719 [49]

### 2.3. Dual MBL and SBL inhibitors

#### 2.3.1. Tricyclic boronic acid derivatives

Compound **58** (reported as a mixture of enantiomers) [50] and a series of *S*-aryl derivatives with **59** as a representative member [51] (Figure 21) were synthesized and evaluated as inhibitors of both MBLs and SBLs. They are structural analogues of xeruboractam (formerly known as QPX7728) [3,4], with variations at the substitution pattern on the aromatic ring. Compound **59** can effectively inhibit all four classes of  $\beta$ -lactamases, i.e. A, B, C, and D (NDM-1, NDM-5, IMP-1, VIM-1, VIM-2, TEM-116, KPC-2, CTX-M-15, AmpC, OXA-10, OXA-48, OXA-23), with  $IC_{50}$  values in the nanomolar range [51]. In combination with cefepime, biapenem, or meropenem, compounds **58** and **59** were able to lower up to 1024-, 1024-, and 64-fold the MIC of *K. pneumoniae*, *E. coli*, and *A. baumannii* producing SBLs and MBLs (KPC-2, OXA-1, OXA-23, NDM-1, NDM-5), respectively [50].

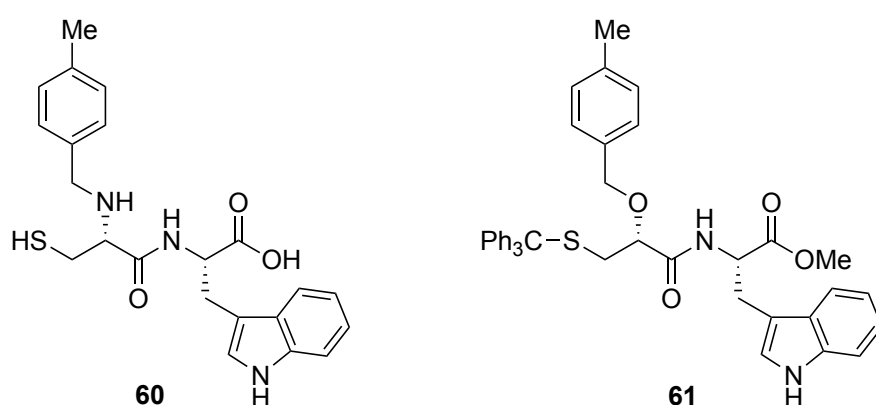


**Figure 21.** Chemical structure of compounds **58** from CN115557978 [50] and **59** from WO2023066292 [51]

#### 2.3.2. Dipeptide-like MBL inhibitors

A series of 28 dipeptide-like compounds was prepared by coupling tryptophan with various derivatives of cysteine. These compounds were evaluated as inhibitors of representative MBLs

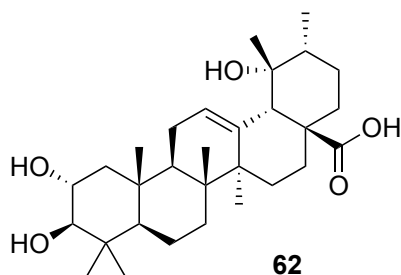
(subclass B1 NDM-1) and SBLs (class A KPC-2 and class D OXA-48) and two different patterns were observed: 19 compounds were active only on NDM-1, with  $IC_{50}$  values between 1.1 and 4.8  $\mu$ M, and the remaining compounds showed significant inhibition of the three enzymes. Representative compounds from the two groups are **60** (Figure 22), with  $IC_{50}$  of 1.8  $\mu$ M against NDM-1, and **61** (Figure 22), with 100, 89, 80 % inhibition at 10  $\mu$ M against NDM-1, KPC-2, and OXA-48, respectively. Interestingly, the thiol and carboxylate groups are not required to be in the free form for optimal inhibition. Moreover, some disulfide dimers obtained by oxidation are also active, against NDM-1 alone or against all three enzymes [52,53].



**Figure 22.** Chemical structure of dipeptide-like  $\beta$ -lactamase inhibitors **60** and **61** from FR3111544 [52] and WO2021255156 [53].

### 2.3.3. Tormentic acid

Tormentic acid (**62**, Figure 23) was reported to inhibit both NDM-1, which is a subclass B1 MBL, and KPC-2, which is a class A SBL, in a dose-dependent manner in the micromolar range [54]. Alone, tormentic acid was not able to influence the normal growth of bacteria, but in combination with imipenem or meropenem could completely kill after 12 h bacterial strains expressing NDM-1 or KPC-2. In an animal model (mouse) infected with an NDM-1-producing *E. coli* strain, the group receiving a combination of tormentic acid and meropenem showed a clear improvement in the survival rate, compared with the groups treated with tormentic acid or meropenem alone, where no obvious treatment effect was observed [54].



**Figure 23.** Chemical structure of tormentic acid **62** from CN113384587 [54]

### **3. Therapeutic potential of metallo- $\beta$ -lactamase inhibitors**

#### 3.1. Combination Therapy

Metallo- $\beta$ -lactamase inhibitors have shown potential when used in combination with  $\beta$ -lactam antibiotics. By inhibiting the MBL activity, these inhibitors restore the efficacy of  $\beta$ -lactams, expanding treatment options for bacterial infections. The patents highlighted in this review and the associated studies have demonstrated synergistic effects between MBL inhibitors and  $\beta$ -lactam antibiotics, leading to possible improvements in the clinical outcomes with pandrug-resistant bacteria.

#### 3.2. Development of dual inhibitors

Dual inhibitors capable of targeting both MBLs and SBLs have gained attention in recent years, and several patents filed in the past 2 years have described the development of such compounds, especially in the cyclic boronate series. The dual inhibitors provide a comprehensive approach to overcoming antibiotic resistance induced by the simultaneous presence of multiple  $\beta$ -lactamases belonging to different Ambler classes, which unfortunately is a situation encountered more and more often in clinical isolates.

### **4. Challenges and future perspectives**

#### 4.1. Resistance development against metallo- $\beta$ -lactamase inhibitors

As with any antimicrobial agent, the development of resistance to MBL inhibitors remains a concern. Bacteria can evolve mechanisms to evade the action of inhibitors, necessitating ongoing surveillance and development of new inhibitors with different selectivity profiles to counter emerging resistance.

#### 4.2. Optimization of inhibitor pharmacokinetics

The pharmacokinetic properties of MBL inhibitors, including their absorption, distribution, metabolism, and excretion, play a crucial role in their therapeutic efficacy. Some patents and associated studies have focused on improving the pharmacokinetic profiles of inhibitors to enhance their bioavailability and tissue penetration.

#### 4.3. Structure-Activity Relationship Studies

Structure-activity relationship (SAR) studies are essential for understanding the interactions between inhibitors and MBLs. Some of the patents presented in this review and the associated studies have utilized SAR studies to optimize the chemical structures of inhibitors and improve their potency, selectivity, and pharmacokinetic properties, in some cases with the help of X-ray crystallography or computational techniques.

### **5. Conclusion**

This patent review has provided a comprehensive analysis of patents filed between January 2020 and May 2023 related to MBL inhibitors, which represent essential tools in overcoming the threat of antibiotic resistance. Recent advancements in the field have demonstrated promising results and highlighted the potential for combination therapies, synergistic effects, and the development of dual inhibitors. However, the development of resistances to current treatments is an important challenge that needs to be addressed, together with a better optimization of the pharmacokinetic and pharmacodynamic properties of the inhibitors to match those of the associated antibiotics.

### **6. Acknowledgments**

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### **Declaration of interests**

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

### **Author contributions**

All authors participated in the conceptualization of the topic, literature searches, writing, and preparation of the manuscript.

### **Expert opinion**

Recent years have witnessed significant advancements in the development of selective MBL inhibitors. The main mechanism of action of these inhibitors is the direct coordination of the zinc ions from the active site. Several interesting families of inhibitors were developed during this period, including 4-amino-1,2,4-triazole-3-thione derivatives, 1-substituted-1*H*-imidazole-2-carboxylic acid derivatives, 3-substituted-1-sulfamoyl-pyrrole-2-carboxylic acid derivatives, and indole-2-carboxylic acid derivatives. On the other hand, a significant number of compounds that inhibit MBLs through a zinc-chelating mechanism were reported. The chelating scaffolds can feature multiple identical pyridyl or carboxylate groups, or a combination of them. In some cases, the zinc-chelating fragment is conjugated with a  $\beta$ -lactam molecule (penicillin, cephalosporin, or carbapenem) to facilitate the targeting and to increase the selectivity of these molecules.

Dual inhibitors, targeting simultaneously both SBLs and MBLs, represent an interesting approach for the treatment of infections related to the presence of multiple  $\beta$ -lactamases from different Ambler classes in the same clinical isolate. The most promising inhibitors with this dual mechanism of action belong to the family of cyclic boronates. In terms of enzyme selectivity, both MBL-specific and dual inhibitors were generally able to inhibit VIM-1, but it was increasingly difficult to develop efficient inhibition against NDM-1, and even more against IMP-1.

MBL-mediated infections in *Pseudomonas aeruginosa* or *Acinetobacter baumannii* were found to be more difficult to treat compared with those in *Enterobacterales*, and an increasing attention should be paid in the future to these clinically relevant pathogens.

Finally, a combined increased effort is expected from the pharmaceutical companies and from the public and private funding bodies for a continuous support of research and development in this critical field of global healthcare and to keep pace with the rapid evolution and worldwide dissemination of antibiotic resistance.

### Article highlights box

- The treatment of infections involving metallo- $\beta$ -lactamases (MBLs) is currently a critical healthcare problem in the absence of any MBL inhibitor approved for clinical use.
- Important progress was made in the development of narrow-spectrum inhibitors targeting MBLs, with either zinc-binding or zinc-chelating mechanisms.
- Large-spectrum dual inhibitors, targeting simultaneously both serine- $\beta$ -lactamases (SBLs) and MBLs, are an interesting approach for the treatment of infections involving multiple  $\beta$ -lactamases.
- Two dual SBL/MBL inhibitors (taniborbactam and xeruborbactam) are currently in clinical trials, and several narrow-spectrum MBL inhibitors are progressing towards clinical development.
- MBL-mediated infections in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are generally more difficult to treat compared to those in *Enterobacteriales*.

### Annotated bibliography

Papers of special note have been highlighted as:

\* of interest

\*\* of considerable interest

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