



HAL
open science

1,2,4-Triazole-3-thione Compounds as Inhibitors of Dizinc Metallo- β -lactamases

Laurent Seville, Laurent Gavara, Carine Bebrone, Filomena De luca, Lionel Nauton, Maud Achard, Paola Mercuri, Silvia Tanfoni, Luisa Borgianni, Carole Guyon, et al.

► **To cite this version:**

Laurent Seville, Laurent Gavara, Carine Bebrone, Filomena De luca, Lionel Nauton, et al.. 1,2,4-Triazole-3-thione Compounds as Inhibitors of Dizinc Metallo- β -lactamases. ChemMedChem, 2017, 12 (12), pp.972-985. 10.1002/cmdc.201700186 . hal-02923462

HAL Id: hal-02923462

<https://hal.science/hal-02923462v1>

Submitted on 19 Dec 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



1,2,4-Triazole-3-thione compounds as inhibitors of di-zinc metallo- β -lactamases

This is the peer reviewed version of the following article:

Original:

Sevaille, L., Gavara, L., Bebrone, C., DE LUCA, F., Nauton, L., Achard, M., et al. (2017). 1,2,4-Triazole-3-thione compounds as inhibitors of di-zinc metallo- β -lactamases. CHEMMEDCHEM, 12(12), 972-985 [10.1002/cmdc.201700186].

Availability:

This version is available <http://hdl.handle.net/11365/1008821> since 2017-05-28T21:43:00Z

Published:

DOI:10.1002/cmdc.201700186

Terms of use:

Open Access

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. Works made available under a Creative Commons license can be used according to the terms and conditions of said license.

For all terms of use and more information see the publisher's website.

(Article begins on next page)

CHEM MED CHEM

CHEMISTRY ENABLING DRUG DISCOVERY

Accepted Article

Title: 1,2,4-Triazole-3-thione compounds as inhibitors of di-zinc metallo- β -lactamases

Authors: Laurent Sevaille, Laurent Gavara, Carine Bebrone, Filomena De Luca, Lionel Nauton, Maud Achard, Paola Mercuri, Silvia Tanfoni, Luisa Borgianni, Carole Guyon, Pauline Lonjon, Gülhan Turan-Zitouni, Julia Dzieciolowski, Katja Becker, Lionel Bénard, Ciaran Condon, Ludovic Maillard, Jean Martinez, Jean-Marie Frère, Otto Dideberg, Moreno Galleni, Jean-Denis Docquier, and Jean-François Hernandez

This manuscript has been accepted after peer review and appears as an Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). This work is currently citable by using the Digital Object Identifier (DOI) given below. The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

To be cited as: *ChemMedChem* 10.1002/cmdc.201700186

Link to VoR: <http://dx.doi.org/10.1002/cmdc.201700186>

WILEY-VCH

www.chemmedchem.org

A Journal of



1,2,4-Triazole-3-thione compounds as inhibitors of di-zinc metallo- β -lactamases

Laurent Sevaille^a, Dr. Laurent Gavara^{a*}, Dr. Carine Bebrone^{b,1}, Dr. Filomena De Luca^c, Lionel Nauton^{d,2}, Dr. Maud Achard^{e,3}, Dr. Paola Mercuri^b, Silvia Tanfoni^c, Luisa Borgianni^c, Dr. Carole Guyon^a, Pauline Lonjon^{a,4}, Dr. Gülhan Turan-Zitouni^f, Dr. Julia Dzieciolowski^g, Prof. Katja Becker^g, Dr. Lionel Bénard^h, Dr. Ciaran Condonⁱ, Dr. Ludovic Maillard^a, Prof. Jean Martinez^a, Prof. Jean-Marie Frère^b, Dr. Otto Dideberg^d, Prof. Moreno Galleni^b, Prof. Jean-Denis Docquier^c and Dr. Jean-François Hernandez^{a*}

^aInstitut des Biomolécules Max Mousseron, UMR5247 CNRS, Université de Montpellier, ENSCM, Faculté de Pharmacie, 15 avenue Charles Flahault, 34093 Montpellier cedex 5, France. ^bLaboratoire de Macromolécules Biologiques, Centre d'Ingénierie des Protéines, Université de Liège, Allée du 6 août B6, Sart-Tilman, 4000 Liège, Belgium. ^cDipartimento di Biotecnologie Mediche, Università di Siena, I-53100 Siena, Italy. ^dInstitut de Biologie Structurale – Jean-Pierre Ebel, UMR5075 CNRS, CEA, Université Joseph Fourier, 41 rue Jules Horowitz, 38027 Grenoble cedex 1, France. ^eEMBL Outstation c/o DESY, Notkestrasse 85, D-22603 Hamburg, Germany. ^fDepartment of Pharmaceutical Chemistry, Anadolu University, Faculty of Pharmacy, 26470 Eskisehir, Turkey. ^gChair of Biochemistry and Molecular Biology, Interdisciplinary Research Center, Justus Liebig University, Heinrich-Buff-Ring 26-32, D-35392 Giessen, Germany. ^hUMR8226, CNRS, Université Pierre et Marie Curie, Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France. ⁱUMR8261, CNRS, Université Paris-Diderot, Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France.

¹Present address: Symbiose Biomaterials S.A., GIGA Bât. B34, 1 avenue de l'Hôpital, 4000 Liège, Belgium.

²Present address: Institut de Chimie de Clermont-Ferrand, UMR6296 CNRS, Université Clermont Auvergne, 63000 Clermont-Ferrand, France.

³Present address: School of Chemistry and Molecular Bioscience, University of Queensland, St Lucia, Brisbane, QLD 4072, Australia.

⁴Present address: CERN, HSE/SEE/SI, CH-1211 Geneva 23, Switzerland.

*Corresponding authors: Tel.: +33-(0)4 11 75 96 03; Fax: +33-(0)4 11 75 96 41.

E-mail addresses: jean-francois.hernandez@umontpellier.fr (J.-F. Hernandez); laurent.gavara@umontpellier.fr (L. Gavara).

Accepted Manuscript

Abstract

Metallo- β -lactamases (MBL) cause resistance of Gram-negative bacteria to β -lactam antibiotics and are of serious concern because they can inactivate the last-resort carbapenems and because MBL inhibitors of clinical value are still lacking. We have previously identified the original binding mode of a 4-amino-1,2,4-triazole-3-thione compound, IIIA, within the di-zinc active site of the L1 MBL. Here, we present the crystallographic structure of a complex of L1 with the corresponding non-amino compound IIIB. Unexpectedly, IIIB showed a similar but reverse binding mode compared to IIIA. These 3D structures suggested that the triazole-thione scaffold was suitable to bind to the catalytic site of di-zinc metallo-enzymes. Based on these results, we synthesized 54 analogues of IIIA or IIIB. Nineteen showed IC_{50} values in the μ M range towards at least one of five representative MBLs (L1, VIM-4, VIM-2, NDM-1, IMP-1). Five of these exhibited a significant inhibition of at least four enzymes, including NDM-1, VIM-2 and IMP-1. Active compounds mainly featured either halogen or bulky bicyclic aryl substituents. Finally, some compounds were also tested on several microbial di-nuclear Zn-dependent hydrolases belonging to the MBL fold superfamily (i.e. endonucleases and glyoxalase II) to explore their activity toward structurally similar but functionally distinct enzymes. While the bacterial tRNases were not inhibited, the best IC_{50} 's towards plasmodial glyoxalase II were in the 10 μ M range.

Introduction

Emergence and spread of antibiotic-resistant bacteria have become an urgent threat to human health due to the emergence of pan-drug resistant Gram-negative pathogens.^[1] In developed countries, the highest proportion of multidrug resistant bacteria is found in hospitals. Indeed, about 70% of bacteria now causing hospital-acquired infections are resistant to at least one of the commonly used drugs. This leads to prolonged illness and increased mortality.

The most worrying resistance concerns the β -lactam antibiotics, the most widely used class of antibacterial agents that includes penicillins, cephalosporins, carbapenems and monobactams.^[2] Among them, carbapenems have the broadest spectrum of activity and represent last resort drugs to treat life-threatening nosocomial infections. These drugs can be inactivated via hydrolysis of their β -lactam ring by bacterial enzymes called β -lactamases. These enzymes are grouped into four molecular classes. Classes A, C, and D act via a serine-based mechanism, whereas class B enzymes, called metallo- β -lactamases (MBLs), require

one or two Zn atom(s) for β -lactam hydrolysis. While combination therapies (treatment with a β -lactamase inhibitor such as clavulanic acid or tazobactam to prevent inactivation of β -lactam antibiotics) are useful to deal with bacteria producing active site serine β -lactamases, MBL inhibitors are not currently available for clinical applications. This is of particular concern because (1) MBLs can degrade all classes of β -lactams (except monobactams), including carbapenems, which are stable towards the large majority of serine- β -lactamases; (2) MBLs are produced by important Gram-negative pathogens (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Aeromonas*, *Enterobacteriaceae*, *Acinetobacter*, *Serratia marcescens*, etc...); (3) many MBL genes are also present in environmental species constituting reservoirs of β -lactam resistance genes. MBLs are now regarded as a major therapeutic challenge.^[3-5]

MBLs are classified into three subclasses, B1, B2, and B3, based on subtle but significant variations in their active site architectures.^[3,4,6-9] Subclass B1 includes the most clinically-relevant acquired MBLs (e.g., IMP-, VIM- and NDM-types). Subclass B2 is represented by CphA and includes enzymes produced by different species of *Aeromonas*, while subclass B3 contains L1, GOB-1, FEZ-1 and other mostly resident enzymes, except AIM-1 and SMB-1 apparently encoded by mobile genetic elements. Subclasses B1 and B3 enzymes have a broad substrate profile and generally exhibit maximum activity as di-zinc species, whereas subclass B2 MBLs are mono-zinc enzymes and narrow-spectrum carbapenemases, with poor or no activity towards penicillins and cephalosporins.

This large structural and functional heterogeneity makes the design of potentially clinically-useful and broad-spectrum MBL inhibitors very challenging.^[For review:10-13] Furthermore, the usual approach to inhibit metallo-enzymes is to develop compounds possessing a zinc-coordinating group (e.g., thiol group). This approach can only have a clinical application when the inhibitor is highly MBL specific (i.e. does not inhibit human metallo-enzymes). To date, several chemical families have been reported to inhibit MBLs. They include biphenyltetrazoles,^[14] various thiol- or thioester-containing compounds,^[15-27] di-^[28-31] or poly-carboxylate compounds,^[32-34] hydroxamates,^[35] arylsulfonyl-containing compounds,^[36-40] trifluoromethyl alcohol and ketones,^[41] tricyclic natural compounds^[42] and cyclic boronates.^[43] Approaches to covalent MBL inhibitors have also been considered.^[19,44,45]

Nevertheless, among all these series, only a limited number of compounds are active (K_i values between 10^{-6} and 10^{-8} M) on several MBLs from the three sub-classes, or even on several of the most clinically relevant enzymes, and small and simple molecules with a zinc

coordinating thiol appear among the most promising in terms of affinity.^[21-23] However, their flexibility and the strong Zn-coordinating properties of the thiol group might compromise their development as highly selective MBL inhibitors.

In a previous crystallographic study,^[46] we obtained the structure of the L1 MBL (a di-zinc subclass B3 enzyme from *Stenotrophomonas maltophilia*) in complex with a published inhibitor (XIII,^[47] Scheme 1). Unexpectedly, the enzyme active site contained a fragment (named IIIA, Scheme 1) derived from the hydrolysis of the hydrazone-like bond of XIII. The same structure (PDB code, 2HB9) was obtained after soaking an L1 crystal in a solution of synthetic IIIA. Additionally, the distinctive feature of the complex L1-IIIA was the as yet unreported double coordination of the two Zn ions by two atoms of the 1,2,4-triazole-3-thione moiety (namely N² for Zn1 and S³ for Zn2, Figure 1). It is noteworthy that a similar binding mode was recently demonstrated for a small triazole-thione compound in complex with VIM-2.^[48]

Scheme 1.

Triazole-thiones are commonly reported as synthetic intermediates, and in biological evaluations. While they have been mainly screened for antibacterial, antifungal, anti-inflammatory, and anti-proliferative activity^[49-53] only a few studies describe their use as metallo-enzyme inhibitors such as the di-copper dopamine- β -hydroxylase,^[54] the TNF- α converting enzyme,^[55] ADAMTS-5^[56] and urease.^[57] A few triazole-thione analogues with no amino group at position 4 were reported to be modest inhibitors of the IMP-1 MBL^[58,59] or shown inactive against the CcrA, ImiS and L1 MBLs at 50 μ M.^[60] Other triazole-thione compounds with an alkylated sulphur have also been published more recently,^[61,62] and the structure of the complex formed by one of these compounds with VIM-2 showed that the two zinc atoms were coordinated by the nitrogen atoms at positions 1 and 2 of the heterocycle.^[63]

Following our findings, we investigated the potential of the triazole-thione heterocycle as a scaffold for MBL inhibition. This study describes the synthesis and evaluation of compound IIIA and analogues as inhibitors of MBL enzymes representative of the three subclasses.

Results and Discussion

Binding mode of 1,2,4-triazole-3-thione to di-zinc enzymes: the case of the MBL L1

The 3D crystallographic structures of several important MBLs have been obtained alone, and in complex with an inhibitor, providing critical information on the binding mode of various inhibitors (IMP-1;^[64] VIM-2;^[65] VIM-4;^[66] NDM-1;^[67] CphA;^[68] L1^[46,69]). As mentioned, compound IIIA (**1a**) simultaneously coordinates the two Zn ions of the L1 active site through two atoms of the 1,2,4-triazole-3-thione moiety: N² for Zn1 and S³ for Zn2.^[46] Moreover, an interaction is formed between the 4-amino group and Asp120, one of the Zn2 ligands (Figure 1), the phenyl ring making a weak CH- π interaction with a methyl group of Ile162. To explore the role of the amino group at position 4 of the heterocycle, we have synthesized IIIB (**1b**, see Table 1), which differed from IIIA by the absence of this amino group. The 3D structure of L1 in complex with IIIB was obtained after soaking native L1 crystals in a solution of the inhibitor, as previously described (See Table S1 in Supplementary Information).^[46] The structure (PDB code, 5DPX) showed that IIIB was also able to coordinate the two Zn ions but, surprisingly, with a pattern different from that of IIIA. Indeed, the 1,2,4-triazole-3-thione heterocycle was interacting with the Zn atoms through N⁴ for Zn1 and S³ for Zn2 (Figure 1). IIIA could not adopt a similar binding mode due to the presence of an amino substituent on the N⁴ atom of the triazole ring. The different orientation of the triazole ring resulted in binding of the aromatic group in an opposite area to the enzyme active site, leading to the formation of a strongly stabilizing π -stacking interaction with His118 (Figures 2 and 3). According to the 2Fo-Fc electron density map (Figure 2), the *o*-toluoyl moiety could adopt two different orientations, which resulted from an 180° rotation of the phenyl ring around the C-C bond between the two cycles. In the case of the slightly major orientation shown in Figure 3, the methyl group of compound IIIB (**1b**) was located in a hydrophobic pocket constituted by Pro227 and Phe156. The superimposition of the two complex structures with that of the free L1 showed that side-chain rearrangements occurred in the active site to adapt to the phenyl group of the two compounds. This adaptation could be explained by the high rigidity of the compounds, and by the constraint associated with the simultaneous double coordination (Figure 4). In particular, the side-chain of Tyr33 was completely retracted in the case of IIIA,^[46] while that of Phe156 was more or less shifted by IIIB at the opposite side of the binding site depending on its methyl orientation. This variable motion of the Phe156 side-chain was supported by its poor electron density (Figure 2) and a B-factor higher than average (Figure S1) in 5DPX.pdb structure. The marked movement of

the Tyr33 side-chain required for IIIA binding is probably more demanding than Phe156 shifting and might explain why IIIB did not bind as IIIA. Finally, a striking feature shared by the two structures was the increase in the Zn-Zn distance from 3.5 to 4.6 Å. Such a movement has already been observed, although to a lesser extent, during the hydrolysis of nitrocefin by L1.^[70]

IIIA and IIIB showed modest activities toward L1, IIIA displaying slightly lower affinity than IIIB ($K_i = 108 \pm 9$ and 35 ± 2 μM , respectively), indicating that the 4-amino group was here less favourable for L1 inhibition.

Figure 1.

Figure 2.

Figure 3.

Figure 4A and 4B.

Based on these results that showed the high interest of the triazole-thione nucleus as a ligand for di-Zn MBLs, we started the synthesis of IIIA and IIIB analogues.

Synthesis

Two series of compounds, analogues of IIIA (**1a**) and of its 4-desamino derivative IIIB (**1b**) (Table 1) were prepared as described in Scheme 2. IIIA analogues were synthesized from the corresponding carboxylic acids (R-COOH) in one step (R = alkyl, Beyer's method, Scheme 2A), and in four steps (R = aryl, Scheme 2B).^[72] IIIB analogues were prepared in three steps (Scheme 2C) by reaction of the acyl chloride with thiosemicarbazide followed by cyclization under basic conditions.

Scheme 2.

Most of the synthesized 1,2,4-triazole-3-thione derivatives were substituted at position 5 by a (hetero)aromatic moiety, either directly linked to the heterocycle (**1a**, **1b**, **5-22**) or separated by one (**23a-31**) or two methylene bridge(s) (**32a-44**) and/or oxygen (**45**, **46**).

Starting from di-carboxylic precursors we prepared six molecules containing two 1,2,4-triazole-3-thione moieties (**29-31**, **41a**, **41b**, **44**). Finally, four compounds (**2a**, **2b**, **3**, **4**) displayed an alkyl substituent (CH₃, CF₃, cyclohexyl) at position 5.

Evaluation of the inhibition of purified MBLs

1,2,4-Triazole-3-thiones were first tested at a 100 μ M concentration for their potential inhibition of five representative MBLs, namely L1, VIM-4, VIM-2, NDM-1 and IMP-1. Enzyme assays were performed in 96-well microtiter plates using nitrocefin as the substrate. Its hydrolysis was monitored by following the absorbance variation at 480 nm at 30 °C. IC₅₀'s were then determined for the best compounds (inhibition at 100 μ M > 70%) by fitting raw absorbance data with the four parameters Hill equation describing a sigmoidal dose–response. The inhibitory potencies are listed in Table 1.

Table 1.

In this single-concentration screening assay, the two parent analogues IIIA (compound **1a**) and IIIB (compound **1b**) appeared either inactive or as weak inhibitors (inhibition <60% at 100 μ M). In addition to **1a** and **1b**, few other compounds allowed comparing the activity of IIIA (4-amino) and IIIB (4-H) analogues displaying the same 5-substituent (**14a** and **14b**, **17a** and **17b**, **23a** and **23b**, **32a** and **32b**, **34a** and **34b**, **41a** and **41b**). Overall, the enzymes did not show any specific preference for a particular series.

Analogues with small alkyl substituents at position 5 (compounds **2a** and **2b** with a methyl group and compound **3** with a trifluoromethyl group), which were prepared to assess the contribution of the 1,2,4-triazole-3-thione to the binding, were also poorly active. These results demonstrated that, while essential for targeting the Zn-containing active sites, the heterocyclic scaffold had very low affinity by itself. Compound **4** with a larger alkyl substituent (cyclohexyl) did not show significant activity either.

Compounds possessing an aryl substituent at position 5 could be divided in three series according to the distance between the triazole-thione ring and the substituent. Overall, aryl compounds having IC₅₀'s in the 1-50 μ M range toward at least one MBL were obtained in the three series.

In the case of the series with an aromatic group directly linked to the triazole, poor inhibition was measured for compound **5** (phenyl), the close analogue of IIIA, and for more

hydrophilic analogues. Indeed, compounds **6** and **7** possessing a phenyl substituted by one or two hydroxyl groups, respectively, were only moderate inhibitors, with the exception of **6**, which inhibited VIM-4 with an IC_{50} of 44 μ M. Furthermore, changing the phenyl ring to the more hydrophilic pyridine (**20**, **21**) or pyrazine (**22**) was not favourable at all.

More interesting results were obtained when the aromatic ring was substituted with either halogens or with a second either fused or separated cyclic moiety. Indeed, with the exception of compound **10** (2-bromophenyl), all analogues bearing a halogen in position 3 or 4 of the phenyl ring (**8**, **9**, **11-13**) inhibited IMP-1 in the μ M range (IC_{50} of 1.7 μ M for **11** to 25 μ M for **13**). Some of them (**9**, **11**, **13** with Cl, Br and CF_3 in *meta* position, respectively) were also active toward NDM-1 (IC_{50} 's between 10 and 30 μ M). Interestingly, the inhibition potency on IMP-1 and NDM-1 seemed to be correlated to the size of the halogen atom, the 3-bromo-phenyl analogue **11** being more favourable than the 3-chloro- and 3-fluoro- derivatives (**9**, **12**). Furthermore, bicyclic analogues of IIIA (**14a** and **15**, 1- and 2-naphthyl, respectively; **16**, indole; **17a**, *p*-benzyloxyphenyl; **18** and **19**, *o*- and *m*-biphenyl, respectively) and IIIB (**17b**, *p*-benzyloxyphenyl; but not **14b**, 1-naphthyl) also significantly inhibited IMP-1 (IC_{50} of 1.4 μ M for **16** to 75 μ M for **18**). Interestingly, compounds **16** (indole), **17a** and **17b** (*p*-benzyloxyphenyl) and **19** (*m*-biphenyl) showed wide activity spectra as they significantly inhibited at least three out of the five tested MBLs. In particular, compounds **16**, **17b** and **19** inhibited the three most clinically relevant MBLs, i.e. NDM-1, VIM-2 and IMP-1, with IC_{50} 's below or close to 50 μ M.

Similar trends were observed when a CH_2 group was introduced between the aromatic ring and the 1,2,4-triazole-3-thione scaffold. Indeed, the simple arylmethyl compounds displaying a benzyl (**23a**, **23b**) or a thienylmethyl (**25**) group, as well as a more hydrophilic *o*-carboxybenzyl substituent (**24**) showed either no or moderate activity toward all enzymes. Again, replacing the phenyl group by a bulkier and hydrophobic bicyclic moiety like a 5,6,7,8-tetrahydronaphthylmethyl (**26**) or a naphthylmethyl (**27**), increased the inhibitory potency as well as the activity spectrum. However, compound **28** with an indolylmethyl group was either an inactive or only a moderate inhibitor of the tested MBLs, indicating that separation of the indole group from the triazole cycle by one CH_2 and/or the substituted position (comparing with **16**), was detrimental for the recognition by all enzyme active sites. In the phenylethyl series, extension of the chain by a second CH_2 group was mainly beneficial to IMP-1 inhibition. Whereas the simple phenylethyl analogue of IIIA (**32a**) was poorly active, its IIIB counterpart **32b** was the best in this series, exhibiting an IC_{50} against IMP-1 in

the μM range (5.4 μM). A similar activity against IMP-1 was measured for compound **33** ($\text{IC}_{50} = 8.1 \mu\text{M}$), the unsaturated analogue of **32b**. Compound **39** with a 4-(trifluoromethyl)phenethyl moiety was poorly active against all enzymes. Bicycle-containing analogues were also significant inhibitors of IMP-1, albeit at a lower level: **34a** (but not **34b**) with a benzodioxole, **40** with an (1*H*-indol-3-yl)ethyl and **42** with a branched 2,2-diphenylethylene exhibited IC_{50} 's in the μM range. Compounds **34a** and **40** also inhibited NDM-1 with an IC_{50} below 50 μM and moderately VIM-2 and VIM-4. As observed in other series, a more polar chain like 4,(3)-(di)hydroxyphenethyl (**35** and **36**), 3,4,5-trimethoxyphenethyl (**37**), 4-(3-carboxy-propion-1-yl)phenethyl (**38**) and 2-amino-2-phenylethyl (**43**), was detrimental for inhibition of all enzymes.

In a closely related series ($\text{CH}_2\text{-CH}_2$ replaced by O-CH_2), the two aryloxymethyl analogues **45** and **46** showed opposite behaviours. Whereas the naphthyloxy-containing compound **46** was found to be poorly active on all enzymes, the phenyloxy derivative **45** showed moderate inhibitory potencies toward VIM-4, VIM-2, NDM-1 and IMP-1. This is a surprising result as the closest analogue of **45**, compound **32a**, was poorly active, and the presence of a bulky aromatic substituent was found favourable in other series. This result suggested that the oxygen atom replacing the CH_2 group in **32a** significantly changed the conformation and/or contributed to hydrogen bonding with MBL active site residues.

Finally, compounds with a double triazole-thione head, and belonging to the benzyl and phenylethyl series (**29-31**, **41a**, **41b** and **44**) were inactive or only moderate inhibitors of all tested MBLs. These results were not that surprising since most of the active compounds in the present study possessed bulky and hydrophobic moieties at position 5 of the triazole-thione heterocycle, and the second triazole-thione moiety could be considered as a polar substituent.

Some compounds were tested against the B2 enzyme CphA (**4**, **7-9**, **13**, **14a**, **14b**, **17a**, **17b**, **20-22**, **24-26**, **28-31**, **33-46**). Most of them showed less than 50% inhibition at 100 μM . Only **26**, **28** and **37** significantly inhibited CphA with IC_{50} values of 25, 30 and 33 μM , respectively, indicating that triazole-thione compounds could also inhibit this mono-zinc MBL.

Overall, compounds with either large aromatic or halogenated aromatic substituents and no hydrophilic group were the most potent inhibitors found in this study. These results were reflected by in silico parameters calculated for all analogues (Table S3). Indeed, compounds with the largest Polar Surface Area ($\text{PSA} \geq 200 \text{ \AA}^2$) were found inactive, with the

exception of compounds **16**, **34a** and **40**, whereas a $\text{LogP} \geq 1.2$ was calculated for all compounds showing a significant activity toward at least one MBL. This was not unexpected since MBL inhibitors generally feature a hydrophobic moiety, which binds in a predominantly hydrophobic pocket of the MBL binding sites. Moreover, the majority of the significant inhibitory potencies were obtained against IMP-1 with nineteen compounds (**8**, **9**, **11-14a**, **15-17b**, **19**, **26**, **27**, **32b-34a**, **40**, **42**, **45**) showing IC_{50} 's below 30 μM and ten (**11**, **15-17b**, **19**, **26**, **32b**, **33**, **42**) below 10 μM (compounds **11**, **16**, **32b**, **33** showed ligand efficiencies (LE) ≥ 0.5 , see Table S4 in Supporting Information). A summary of SAR for IMP-1 is presented in Figure 5. Among these compounds, some showed wider spectra of activity toward NDM-1 (**9**, **11**, **13**, **14a**, **16**, **17b**, **19**, **26**, **34a**, **40**, **45**) and VIM-2/VIM-4/L1 (**16**, **17a**, **17b**, **19**, **26**, **27**, **45**). It is noteworthy that none of the halogenated compounds significantly inhibited VIM-2. These different behaviours between the B1 enzymes IMP-1, NDM-1 and VIM-2 probably reflect the variability in the structure of the loops involved in inhibitor binding.^[73] In this context, it is not surprising that VIM-2 and VIM-4 here shared a very close inhibition profile. Finally, compounds **16** (indolyl), **17b** (benzyloxyphenyl), **19** (*m*-biphenyl), **26** (5,6,7,8-tetrahydronaphthylmethyl) and **27** (naphthylmethyl) were the most interesting MBL inhibitors reported in this study.

Figure 5.

Evaluation of triazole-thione analogues as inhibitors of other di-zinc enzymes of the MBL superfamily

MBLs belong to the so-called MBL superfamily, which contains numerous other binuclear Zn-dependent hydrolases with similar protein folds but exhibiting different biological functions, such as glyoxalase II, several nucleases, the quorum-quenching N-acyl-L-homoserine lactone hydrolase, or the bacterial desuccinylase DapE.^[3,74] We evaluated the potential inhibitory effect of some of the triazole-thione compounds on three of these enzymes: glyoxalase II from *Plasmodium falciparum* (Table 2), and the exo/endoribonucleases tRNase Z from *Escherichia coli* and RNase J1 from *Bacillus subtilis*. In the case of the ribonucleases, none of the tested compounds (**1a**, **1b**, **2a**, **2b**, **3**, **5**, **23a**, **23b**, **32a**, **32b**) showed inhibitory activity against RNase J1 at 100 μM or against tRNase Z at 1 mM, with the exception of the IIIA analogue **2a**, which had a K_i of 0.2 mM against tRNase Z. Since this compound possesses a methyl group, the smallest substituent in the series, these

results suggested that a bigger substituent at this position could not be accommodated by the enzyme. The corresponding non-amino compound **2b** was inactive. Therefore, the 1,2,4-triazole-3-thione scaffold did not appear to be suitable for the inhibition of di-zinc ribonucleases.

A representative panel of triazole-thione compounds (**1a-5**, **7**, **14a**, **17a**, **23a-23b**, **25**, **26**, **28**, **29**, **32a-36**, **38a**, **38b**, **40**, **41b**, **43-45**) was tested on glyoxalase II from *Plasmodium falciparum* (Table 2). Few of them showed modest inhibition with IC₅₀'s in the 10-60 μM range, the best inhibitor being compound **1a** (IIIA). When comparison was possible, the 4-amino derivatives were generally more potent than their non-amino counterparts (**1a/1b**, **2a/2b**, **23a/23b**, **32a/32b**, **34a/34b**).

Table 2.

Conclusion

Overall this study confirms the potential of the 1,2,4-triazole-3-thione moiety (2,4-dihydro-3H-1,2,4-triazole-3-thione) as a metal ligand, well adapted to target the di-zinc active sites of metallo-β-lactamases. 54 analogues of compounds IIIA (**1a**) and IIIB (**1b**) differing on their side chain at position 5 of the heterocycle were prepared. We first demonstrated that L1, VIM-4, VIM-2, NDM-1 and IMP-1 did not show any specific preference for one of the 4H-1,2,4-triazole-3-thione or 4-amino-1,2,4-triazole-3-thione series. By exploring the pattern at position 5, we identified several inhibitors with an IC₅₀ in the micromolar range toward at least one MBL (either L1, VIM-4, VIM-2, NDM-1 or IMP-1). Of high interest, several compounds significantly inhibited the three most clinically-relevant MBLs, i.e. NDM-1, IMP-1 and VIM-2. In particular, the inhibitor **16** showed IC₅₀s below 50 μM toward these three enzymes as well as against VIM-4 and L1 (Table 1). This indicated that broad-spectrum inhibitors could be obtained from this series. In addition, a few compounds showed significant inhibitory activity toward the B2 enzyme CphA, suggesting that the heterocycle-thione could also target mono-zinc MBLs.

Screening some compounds against other enzymes of the MBL superfamily showed that triazole-thione analogues were non-functional for ribonucleases, which do not accept substituents larger than a methyl group at the 5-position. Higher activity was observed against the *Plasmodium* glyoxalase II. The size of the triazole-thione moiety, the constraint associated

to the double coordination, and the moderate affinity for metals resulting in a very low potency for the small analogues **2a-3** might offer a greater probability of sparing human metallo-enzymes. Based on these results, further developments toward more potent triazole-thione analogues are in progress.

Experimental section

1) Chemistry

General Methods

NMR. ^1H NMR and ^{13}C NMR spectra were recorded on a 300 or 400 MHz instrument in DMSO-*d*₆ solutions unless otherwise indicated. Splitting patterns in ^1H NMR spectra were designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad.

Reverse phase HPLC. Samples were prepared in an acetonitrile/water (50/50 v/v) mixture. Compounds insoluble in this mixture were solubilized in DMSO. RP HPLC analyses were performed on a Chromolith SpeedRod C18 column (0.46 x 5 cm) by means of a linear gradient (0-100%) of 0.1% TFA/acetonitrile in 0.1% aqueous TFA over 5 min, and at a flow rate of 3 mL/min. **LC-MS.** The LC/MS system consisted of a Waters Alliance 2690 HPLC, coupled to a Waters-Micromass ZQ spectrometer (electrospray ionization mode, ESI+). All analyses were carried out using a RP C18 monolithic Onyx Phenomenex 2.5 x 0.46 cm column. The flow rate was set to 3 mL/min with eluent A (water/0.1% formic acid) and a gradient of 0% → 100% of eluent B (acetonitrile/0.1% formic acid) over 3 min was then used. Positive ion electrospray mass spectra were acquired at a solvent flow rate of 100–500 $\mu\text{L}/\text{min}$. Nitrogen was used as both the nebulizing and drying gas. The data were obtained in a scan mode in 0.1s intervals; 10 scans were summed up to get the final spectrum. **TLC.** Thin-layer chromatography was performed on aluminium-backed sheets of silica gel F₂₅₄ (0.2 mm), which were visualised under 254 nm light, and by spraying with a 2% EtOH solution of ninhydrin followed by heating, or by charring with an aqueous solution of ammonium sulphate and sulfuric acid (200 g (NH₄)₂SO₄ and 40 mL concentrated sulfuric acid in 1 L of water). **Purification.** Column chromatographies were performed using Merck silica gel 60 of particle size 40-63 μm .

(i) General procedures for the preparation of 4-amino-5-substituted-triazole-3-thiones

- Route A (R ≠ aryl)

The carboxylic acid compound and thiocarbohydrazide (1 or 2 equiv.) were ground up and mixed together in a mortar, then heated between 140 and 160 °C until fusion occurred. After

cooling down, a dilute aqueous solution of Na_2CO_3 was added and the mixture was heated at 70 °C for 30 min. Generally, a precipitate appeared upon cooling, which was collected by filtration and washed with water. Otherwise, water was evaporated. Compounds were either purified by crystallization in ethanol or on a silica gel column.

- Route B (R = aryl)

It has to be noted that hydrazine and carbon disulphide used during this procedure have to be handled with caution.

The carboxylic compound was first converted into its ethyl ester by refluxing in absolute ethanol in the presence of a few drops of H_2SO_4 . The ester was then treated overnight with hydrazine hydrate (2 to 4 equiv.) without solvent at 120 °C. Evaporation of excess hydrazine gave the corresponding hydrazide compound. The hydrazide, solubilised in absolute ethanol, was treated with CS_2 (5 equiv.) in the presence of KOH (1.7 equiv.) at 85 °C for 3 h. Water was added and pH was adjusted to 2-3 with 1N HCl. The formed precipitate was collected by filtration and washed with water, yielding the 1,3,4-oxadiazol-thione, which was used without further purification. Finally, the preceding compound was treated with hydrazine hydrate (10 equiv.) in absolute ethanol at 100 °C overnight in a sealed tube. After evaporation of excess hydrazine, the residue was purified on a silica gel column to yield the final compound.

(ii) General procedure for the preparation of non-amino 5-substituted-triazole-3-thiones

To a solution of the corresponding carboxylic acid in toluene (5 mL per gram), 10 equivalents of thionyl chloride were added and the mixture was refluxed for 5 h. After removal of the solvent under vacuum, the residue was taken up in toluene (20 mL per gram) and the solution was added to a suspension of 1 equivalent of thiosemicarbazide in pyridine (20 mL per gram) cooled in an ice/salt bath. The mixture was stirred at -5 °C for 1 h and at RT overnight. After filtration, the solvent was evaporated, and a 2 N aqueous NaOH solution (50 mL per gram) was added. The mixture was refluxed for 4 h and acidified to pH 2 with concentrated aqueous HCl. The formed precipitate was then recovered by filtration, washed with 0.1 N HCl and purified by chromatography on a silica gel column.

Physical data and spectral information for all compounds are provided in the Supporting Information.

2) Enzymology

a) Metallo- β -Lactamase inhibition assay

The 1,2,4-triazole-3-thione compounds were screened for inhibition against six representative metallo- β -lactamases, namely the acquired IMP-1, VIM-2 and VIM-4 and NDM-1, CphA from *Aeromonas hydrophila* and L1 from *Stenotrophomonas maltophilia*. Purified preparations of these enzymes were already available and were used in enzyme assays. Buffers were prepared with Milli-Q water and ZnII residual concentrations were below 0.4 μ M.

IMP-1, VIM-2, VIM-4, NDM-1, L1

Stock solutions: for NDM-1, VIM-2, VIM-4, IMP-1 and L1 assays, nitrocefin was solubilized at 10 mM in DMSO then diluted at 128 μ M, 400 μ M, 480 μ M, 720 μ M and 64 μ M respectively in 50 mM HEPES buffer solution pH 7.0 containing 50 μ M ZnSO₄. The 1,2,4-triazole-3-thiones were solubilized at 10 mM in DMSO then diluted at 133 μ M in the same buffer. NDM-1, VIM-2, VIM-4, IMP-1 and L1 enzymes were diluted at 80, 0.8, 0.8, 48 and 48 nM respectively in 50 mM HEPES buffer solution pH 7.0 containing 50 μ M ZnSO₄ and 20 μ g/mL BSA.

Enzyme inhibition assays were performed in non-sterile acrylic 96-well microtiter plates with UV transparent flat bottom (Corning) in a reaction volume of 200 μ L. Final DMSO concentration in the assay did not exceed 1%. The enzymes (25 μ l stock solution) were mixed with 1,2,4-triazole-3-thiones (150 μ l) so that the final concentration of compound reached 100 μ M. Pre-incubation was performed at 30°C for 30 min then the reaction was initiated by addition of nitrocefin solution (25 μ l). Nitrocefin final concentration was 16, 50, 60, 90 and 8 μ M for NDM-1, VIM-2, VIM-4, IMP-1 and L1, respectively, corresponding to 3 to 4 times the K_m (measured K_m values were $4.4 \pm 0.5 \mu$ M, $16.5 \pm 0.8 \mu$ M, $18.9 \pm 0.4 \mu$ M, $31 \pm 10 \mu$ M and $2.0 \pm 0.3 \mu$ M for NDM-1, VIM-2, VIM-4, IMP-1 and L1, respectively). NDM-1, VIM-2, VIM-4, IMP-1 and L1 final concentrations were 10, 0.1, 0.1, 0.6 and 0.6 nM, respectively. Nitrocefin hydrolysis was monitored at 30°C by following the absorbance variation at 480 nm using a Victor 3V microplate reader. Initial velocities were obtained from plots of absorbance versus time, using data points from the linear portion of the hydrolysis curve. Inhibition percentages were calculated from the ratio of initial velocities with and without inhibitor. IC₅₀ values were determined for compounds exhibiting >70% inhibition at 100 μ M. Final concentrations of 1,2,4-triazole-3-thiones were adjusted to 0-200 μ M by 1:2 serial dilutions of

the stock solutions. IC_{50} 's were determined by fitting raw absorbance data with the four Hill equation parameters describing a sigmoidal dose–response curve using Microsoft Office Excel 2016. The assays were performed in triplicates; Errors were less than 5%.

The quality of these enzyme assays was determined by calculating their Z' factors^[75] with the following equation:

$$Z' \text{ factor} = 1 - \left(\frac{(3\sigma_p + 3\sigma_n)}{|\mu_p - \mu_n|} \right)$$

where σ is the standard deviation and μ is the mean initial velocity for positive (p) and negative (n) controls. Z' factors for NDM-1, VIM-2, VIM-4, IMP-1 and L1 inhibition tests were found to be 0.70, 0.70, 0.79, 0.62 and 0.74, respectively.

CphA

Compounds were prepared as 10 mM solutions in DMSO before dilution with 15 mM Cacodylate pH 6.5 buffer for CphA. The enzyme was used at a final concentration of 0.8 nM. The enzyme and the inhibitor (100 μ M) were pre-incubated for 30 min in a volume of 495 μ L at 21°C. Then, 5 μ L of 10 mM imipenem were added and the hydrolysis of imipenem was monitored by following the absorbance variation at 300 nm, using an UvikonXL spectrophotometer connected to a computer via an RS232 serial interface. Experiments were performed at 30 °C and initial rate conditions were used to study the inhibition with imipenem. The activity was tested by measuring the initial rates in three samples without inhibitor, which allowed determining the percentage of residual β -lactamase activity in the presence of inhibitors. When the residual activity in the presence of 100 μ M inhibitor was < 30%, the initial rate conditions were used to study the inhibition in the presence of increasing concentrations of compounds (from 1 to 100 μ M) and to determine the IC_{50} values. The assays were performed in triplicate; Errors were less than 5%.

b) Test of RNase J1 ribonuclease activity

B. subtilis RNase J1 was pre-incubated with inhibitors for 10 min on ice in 10 μ L reaction buffer (20 mM Tris-HCl pH 8.0, 8 mM $MgCl_2$, 100 mM NH_4Cl , 0.1 mM DTT). The reaction was started by adding 10 μ L quenched RNA (ACUAGA-FAM-CAUACA-TAMRA) in the same buffer. The enzyme was assayed at 0.3 μ M and inhibitors were tested at 10, 20, and 100 μ M final concentration. Release of FAM (fluorescein amidite) fluorescence (520 nm)

following cleavage between FAM and TAMRA (tetramethylrhodamine) moieties was assayed at 30 °C in a Rotor Gene RT-PCR apparatus as described.^[76]

c) Kinetic measurements with *E. coli* tRNase Z

The *E. coli* tRNase Z was purified as previously described.^[77] Phosphodiesterase activity was measured using bis(*p*-nitrophenyl)phosphate (bpNPP) as a substrate.^[78] Reactions were performed in 20 mM Tris HCl pH 7.4 100 mM NaCl at 22 °C. After a 15 min incubation of tRNase Z with the inhibitor, reactions were initiated by adding bpNPP and monitored by measuring the release of *p*-nitrophenol (pNP) at 405 nm for 2 min. Catalytic activities were expressed in μmol of pNP generated per minute per active subunit at 22 °C. All measurements were performed in triplicates and experiments repeated with at least two independent enzyme preparations.

d) Test of *Plasmodium falciparum* cytosolic glyoxalase II (PfcGloII) activity

The recombinant enzyme was produced in *E. coli* and purified as described.^[79] For measuring GloII activity, the decrease in absorbance resulting from *S*-D-lactoylglutathione ($\epsilon_{240\text{nm}} = 3.1 \text{ mM}^{-1}\cdot\text{cm}^{-1}$) hydrolysis was measured at 25 °C in 100 mM 4-morpholinopropane sulfonate (MOPS) buffer, pH 7.2 in a total volume of 0.5 mL. *S*-D-lactoylglutathione was obtained from Sigma. Inhibitors were dissolved in DMSO and added to the enzyme assay mixture before the reaction was started with enzyme (5-25 nM PfcGloII). For each assay background absorbances induced by the inhibitors were taken into account. All experiments were carried out in duplicates. Results are provided as mean values that differed by less than 15%.

Acknowledgements

We thank Mr Pierre Sanchez for mass spectrometry analyses and Wolfram Meyer-Klaucke for advice about tRNase Z. Part of this work was supported by *Agence Nationale de la Recherche* (ANR-14-CE16-0028-01, including fellowship to L.S.), the *Deutsche Forschungsgemeinschaft* (BE1540/15-2 within SPP 1710 to K.B.) and *Agence Nationale de la Recherche* (“subtilRNA”) to C.C.

Legends to Schemes and Figures.

Scheme 1. Hydrolysis of compound XIII^[47] into compound IIIA^[46] and 2-carboxy-benzaldehyde in equilibrium with the cyclic lactol.

Scheme 2. Synthesis of 1,2,4-triazole-3-thione derivatives. **A.** 4-amino derivatives with R ≠ Aryl. **B.** 4-amino derivatives with R = Aryl (Ar). **C.** 4-H derivatives. *Reagents and conditions:* (a) neat, 160 °C; (b) hydrazine hydrate, neat, 120 °C, sealed tube; (c) CS₂, KOH, EtOH, reflux; (d) hydrazine hydrate, EtOH, 100 °C, sealed tube; (e) SOCl₂, toluene, reflux; (f) pyridine, toluene, RT; (g) aqueous NaOH, reflux.

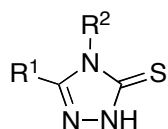
Figure 1.^[71a] Binding mode of the 1,2,4-triazole-3-thione moiety of compounds IIIA (**1a**) and IIIB (**1b**) in the di-zinc active site of L1. The structure of the complex L1/IIIA, 2HB9.pdb was reported in reference [46].

Figure 2.^[71b] Electron density map for the structure of compound IIIB (**1b**) binding in L1 active site (5DPX.pdb). In blue, 2Fo-Fc map at 1.2 sigma contour level; in green and red, Fo-Fc map at 3.5 sigma contour level.

Figure 3.^[71b] Compound IIIB (**1b**) in the L1 active site from 5DPX.pdb. The image shows the interactions established with the two Zn ions and the π-stacking with His118.

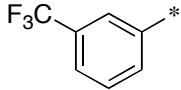
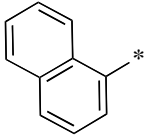
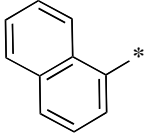
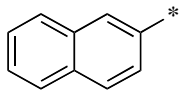
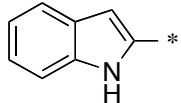
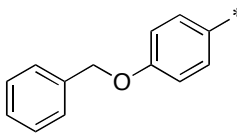
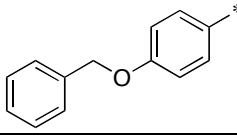
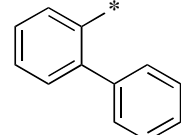
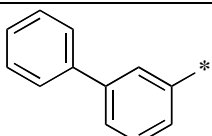
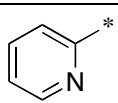
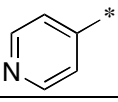
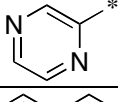
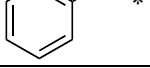
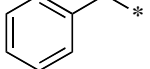
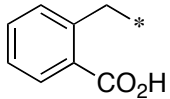
Figure 4.^[71b] Superimposition of the 3-D structures of free L1 (green side-chains for Tyr33 and Phe156, from 2FM6.pdb^[46]) and L1 complexed to (A) IIIA (blue side-chains, from 2HB9.pdb^[46]) and (B) IIIB (purple side-chains, from 5DPX.pdb). The carbon atoms of the inhibitors are shown in orange. The Zn ions are depicted in magenta.

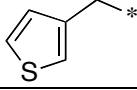
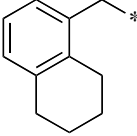
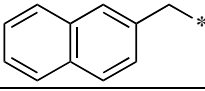
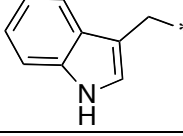
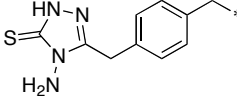
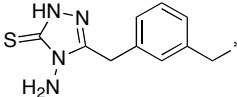
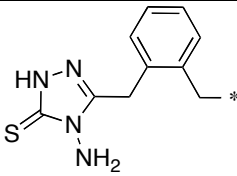
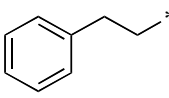
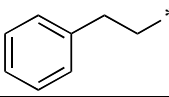
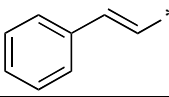
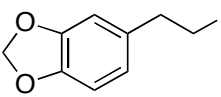
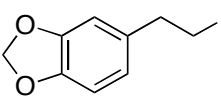
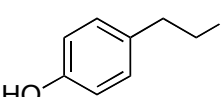
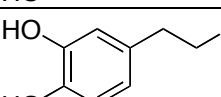
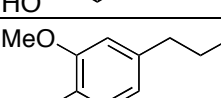
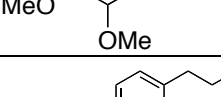
Figure 5. Summary of SAR of 1,2,4-triazole-3-thione derivatives for IMP-1 inhibition. These SAR are valid for all enzymes excepted that VIM-2, VIM-4 and L1 are not or only poorly inhibited by halogenated analogues.

Table 1. Inhibitory activity of 1,2,4-triazole-3-thiones against various MBLs.

Cpd	Structure		IC ₅₀ (μM) or (% inhibition at 100 μM) ^a				
	R ¹	R ²	L1	VIM-4	VIM-2	NDM-1	IMP-1
1a (IIIA)		NH ₂	(24%)	NI	NI	(20%)	NI
1b (IIIB)		H	(23%)	NI	NI	(18%)	(57%)
2a	CH ₃ -	NH ₂	NI	NI	NI	(11%)	NI
2b	CH ₃ -	H	NI	NI	(36%)	(21%)	(45%)
3	CF ₃ -	NH ₂	NI	NI	NI	NI	NI
4		NH ₂	NI	NI	NI	(47%)	NI
5		NH ₂	NI	NI	(13%)	NI	(48%)
6		NH ₂	(14%)	44.0±0.6	(13%)	(57%)	(41%)
7		NH ₂	(45%)	(29%)	NI	(37%)	(≤10%)
8		NH ₂	(31%)	(33%)	(16%)	(36%)	13±1
9		NH ₂	(27%)	(31%)	(27%)	24±1	18±4
10		NH ₂	(29%)	NI	NI	(56%)	NI
11		NH ₂	NI	(53%)	(53%)	13±1	1.7±0.2
12		NH ₂	NI	(12%)	(14%)	(18%)	13±3

Accepted Manuscript

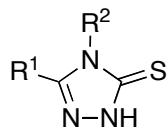
13		NH ₂	(20%)	(38%)	(40%)	29±2	25±0.3
14a		NH ₂	(60%)	101±2	(38%)	36±1	23±1
14b		H	NI	(45%)	NI	(41%)	(43%)
15		NH ₂	(11%)	NI	NI	(16%)	8.1±0.2
16		NH ₂	32±1	19±2	14±1	34±3	1.4±0.1
17a		NH ₂	(43%)	14±2	17±1	(52%)	7.2±0.3
17b		H	97±13	25±1	49±6	11±1	1.8±0.2
18		NH ₂	5.3±0.5	60±5	63±1	104±11	75±18
19		NH ₂	(16%)	44±2	51±1	13±1	5.6±0.3
20		NH ₂	NI	NI	NI	NI	NI
21		NH ₂	NI	NI	NI	NI	NI
22		NH ₂	NI	NI	NI	NI	NI
23a		NH ₂	(45%)	(14%)	NI	(53%)	NI
23b		H	(18%)	NI	NI	(24%)	(66%)
24		NH ₂	(31%)	NI	NI	NI	NI

25		NH ₂	(63%)	NI	(11%)	(15%)	(31%)
26		NH ₂	(23%)	46±1	49±2	12±1	5.0±0.2
27		NH ₂	33±2	31±2	36±1	54±16	11±1
28		NH ₂	NI	(14%)	NI	(27%)	(60%)
29		NH ₂	(62%)	(28%)	NI	(41%)	(33%)
30		NH ₂	(25%)	NI	NI	(27%)	NI
31		NH ₂	NI	NI	NI	NI	(23%)
32a		NH ₂	NI	(23%)	(27%)	(14%)	(45%)
32b		H	NI	NI	NI	(34%)	5.4±0.9
33		H	NI	(12%)	NI	(27%)	8.0±0.8
34a		NH ₂	NI	(44%)	(41%)	44±3	27±5
34b		H	NI	NI	NI	(44%)	(53%)
35		H	NI	NI	NI	NI	(42%)
36		NH ₂	NI	NI	NI	NI	NI
37		NH ₂	NI	NI	NI	NI	NI
38b		H	NI	NI	NI	NI	NI

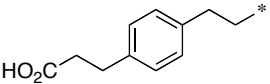
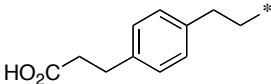
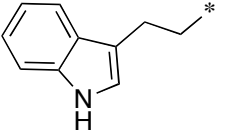
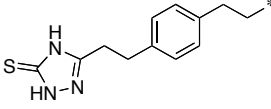
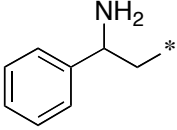
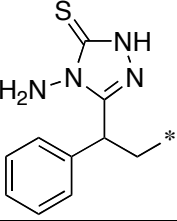
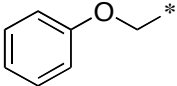
39		NH ₂	(26%)	NI	NI	(15%)	(33%)
40		NH ₂	NI	(65%)	(64%)	26±2	14±0.1
41a		NH ₂	(63%)	(33%)	(13%)	NI	(56%)
41b		H	(45%)	NI	NI	(30%)	NI
42		NH ₂	NI	(53%)	(45%)	NI	5.1±0.7
43		NH ₂	(13%)	NI	NI	(20%)	NI
44		NH ₂	(25%)	NI	NI	(15%)	NI
45		NH ₂	(55%)	46±7	39±1	42±3	25±3
46		NH ₂	(32%)	(10%)	(20%)	ND	(26%)

NI: no inhibition ($\leq 10\%$ inhibition at $100 \mu\text{M}$). ND: not determined. ^aAssays performed in triplicates using nitrocefin as substrate after 30 min pre-incubation of the compounds with the enzymes. Kinetic monitored at 30°C by following the absorbance variation at 480 nm. IC_{50} 's were determined when inhibition at $100 \mu\text{M} > 70\%$.

Table 2. Inhibitory activities of 1,2,4-triazole-3-thiones against glyoxalase II from *Plasmodium falciparum*.



Cpd	Structure		IC ₅₀ (μ M)	Cpd	Structure		IC ₅₀ (μ M)
	R ¹	R ²			R ¹	R ²	
1a IIIa		NH ₂	10	1b IIIb		H	22
2a	CH ₃ -	NH ₂	>60 (14%) ^a	2b	CH ₃ -	H	>60 (8%)
3	CF ₃ -	NH ₂	60	4		NH ₂	>40 (10%)
5		NH ₂	22	7		NH ₂	>40 (0%)
14a		NH ₂	>40 (33%)	17a		NH ₂	20
23a		NH ₂	60	23b		H	>40 (2%)
25		NH ₂	>20 (0%)	26		NH ₂	60
28		NH ₂	>40 (0%)	29		NH ₂	>20 (0%)
32a		NH ₂	30	32b		H	>40 (22%)
				33		H	>20 (0%)
34a		NH ₂	19	34b		H	>20 (25%)
35		H	>60 (23%)	36		NH ₂	>20 (0%)

38a		NH ₂	>20 (25%)	38b		H	60
40		NH ₂	>40 (11%)	41b		H	>30 (0%)
43		NH ₂	>40 (0%)	44		NH ₂	>20 (30%)
45		NH ₂	>20 (23%)				

^aPercentage inhibition at the highest tested concentration. Due to the high absorbance of the compounds at 240 nm, only limited concentrations could be tested in the enzymatic assay. For example, > 60 (40%) means that the highest concentration tested was 60 μM; at this concentration the enzyme was inhibited by 40%. Therefore, IC₅₀ > 60 μM.

References

- [1] a) T. R. Walsh, M. A. Toleman, *J. Antimicrob. Chem.* **2012**, *67*, 1-3; b) G. M. Rossolini, F. Arena, P. Pecile, S. Pollini, *Curr. Opin. Pharmacol.* **2014**, *18*, 56-60
- [2] P. Nordmann, *Med. Mal. Inf.* **2014**, *44*, 51-56
- [3] C. Bebrone, *Biochem. Pharmacol.* **2007**, *74*, 1686-1701
- [4] T. R. Walsh, M. A. Toleman, L. Poirel, P. Nordmann, *Clin. Microbiol. Rev.* **2005**, *18*, 306-325
- [5] E. K. Phelan, M. Miraula, C. Selleck, D. L. Ollis, G. Schenk, N. Mitic, *Am. J. Mol. Biol.* **2014**, *4*, 89-104
- [6] V. P. Sandanayaka, A. S. Prashad, *Curr. Med. Chem.* **2002**, *9*, 145-165
- [7] M. Babic, A. M. Hujer, R. A. Bonomo, *Drug Resist. Updates* **2006**, *9*, 142-156
- [8] P. Nordmann, T. Naas, L. Poirel, *Emerging Infectious Diseases* **2011**, *17*, 1791-1798
- [9] T. Palzkill, *Ann. N. Y. Acad. Sci.* **2013**, *1277*, 91-104
- [10] C. Bebrone, P. Lassaux, L. Vercheval, J. S. Sohier, A. Jehaes, E. Sauvage, M. Galleni, *Drugs* **2010**, *70*, 651-669
- [11] S. M. Draws, R. A. Bonomo, *Clin. Microbiol. Rev.* **2010**, *23*, 160-201
- [12] P. Oelschlaeger, N. Ai, K. T. Duprez, W. J. Welsh, J. H. Toney, *J. Med. Chem.* **2010**, *53*, 3013-3027
- [13] Z. Guo, S. Ma, *Curr. Drug Targets* **2014**, *15*, 689-702
- [14] J. H. Toney, P. M. Fitzgerald, N. Grover-Sharma, S. H. Olson, W. J. May, J. G. Sundelof, D. E. Vanderwall, K. A. Cleary, S. K. Grant, J. K. Wu, J. W. Kozarich, D. L. Pompliano, G. G. Hammond, *Chem. Biol.* **1998**, *5*, 185-196
- [15] S. Bounaga, A. P. Laws, M. Galleni, M. I. Page, *Biochem. J.* **1998**, *331*, 703-711
- [16] D. J. Payne, J. H. Bateson, B. C. Gasson, D. Proctor, T. Khushi, T. H. Farmer, D. A. Tolson, D. Bell, P. W. Skett, A. C. Marshall, R. Reid, L. Ghosez, Y. Combret, J. Marchand-Brynaert, *Antimicrob. Agents Chemother.* **1997**, *41*, 135-140
- [17] C. Mollard, C. Moali, C. Papamicael, C. Damblon, S. Vessilier, G. Amicosante, C. J. Schofield, M. Galleni, J.-M. Frère, G. C. Roberts, *J. Biol. Chem.* **2001**, *276*, 45015-45023
- [18] S. Siemann, A. J. Clarke, T. Viswanatha, G. I. Dmitrienko, *Biochemistry* **2003**, *42*, 1673-1683

- [19] H. Kurosaki, Y. Yamaguchi, T. Higashi, K. Soga, S. Matsueda, H. Yumoto, S. Misumi, Y. Yamagata, Y. Arakawa, M. Goto, *Angew. Chem. Int. Ed. Engl.* **2005**, *44*, 3861-3864; *Angew. Chem.* **2005**, *117*, 3929-3932
- [20] Q. Sun, A. Law, M. W. Crowder, H. M. Geysen, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5169-5175
- [21] B. M. R. Liénard, R. Hüting, P. Lassaux, M. Galleni, J.-M. Frère, C. J. Schofield, *J. Med. Chem.* **2008**, *51*, 684-688
- [22] B. M. R. Liénard, G. Garau, L. Horsfall, A. I. Karsisiotis, C. Damblon, P. Lassaux, C. Papamicael, G. C. Roberts, M. Galleni, O. Dideberg, J.-M. Frère, C. J. Schofield, *Org. Biomol. Chem.* **2008**, *6*, 2282-2294
- [23] P. Lassaux, M. Hamel, M. Gulea, H. Delbrück, P. S. Mercuri, L. Horsfall, D. Dehareng, M. Kupper, J.-M. Frère, K. Hoffmann, M. Galleni, C. Bebrone, *J. Med. Chem.* **2010**, *53*, 4862-4876
- [24] X.-L. Liu, Y. Shi, J. S. Kang, P. Oelschlaeger, K.-W. Yang, *ACS Med. Chem. Lett.* **2015**, *6*, 660-664
- [25] F.-M. Klingler, T. A. Wichelhaus, D. Frank, J. C. Bernal, J. El-Delik, H. F. Müller, H. Sjuts, S. Göttig, A. Koenigs, K. M. Pos, D. Pogoryelov, E. Proschak, *J. Med. Chem.* **2015**, *58*, 3626-3630
- [26] O. K. Arjomandi, W. M. Hussein, P. Vella, Y. Yusof, H. E. Sidjabat, G. Schenk, R. P. McGeary, *Eur. J. Med. Chem.* **2016**, *114*, 318-327
- [27] P. Hinchliffe, M. M. Gonzalez, M. F. Mojica, J. M. Gonzalez, V. Castillo, C. Saiz, M. Kosmopoulou, C. L. Tooke, L. I. Llarrull, G. Mahler, R. A. Bonomo, A. J. Vila, J. Spencer, *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 3745-3754
- [28] J. H. Toney, G. G. Hammond, P. M. Fitzgerald, N. Sharma, J. M. Balkovec, G. P. Rouen, S. H. Olson, M. L. Hammond, M. L. Greenlee, Y. D. Gao, *J. Biol. Chem.* **2001**, *276*, 31913-31918
- [29] L. E. Horsfall, G. Garau, B. M. Liénard, O. Dideberg, C. J. Schofield, J.-M. Frère, M. Galleni, *Antimicrob. Agents Chemother.* **2007**, *51*, 2136-2142
- [30] Y. Hiraiwa, A. Morinaka, T. Fukushima, T. Kudo, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5162-5165
- [31] Y. Ishii, M. Eto, Y. Mano, K. Tateda, K. Yamaguchi, *Antimicrob. Agents Chemother.* **2010**, *54*, 3625-3629
- [32] A. M. King, S. A. Reid-Yu, W. Wang, D. T. King, G. De Pascale, N. C. Strynadka, T. R. Walsh, B. K. Coombes, G. D. Wright, *Nature* **2014**, *510*, 503-506

- [33] F. von Nussbaum, G. Schiffer, *Angew. Chem. Int. Ed. Engl.* **2014**, *53*, 11696-11698; *Angew. Chem.* **2014**, *126*, 11884-11887
- [34] A. M. Somboro, D. Tiwari, L. A. Bester, R. Parboosing, L. Chonco, H. G. Kruger, P. I. Arvidsson, T. Govender, T. Naicker, S. Y. Essack, *J. Antimicrob. Chemother.* **2015**, *70*, 1594-1596
- [35] B. M. R. Liénard, L. E. Horsfall, M. Galleni, J.-M. Frère, C. J. Schofield, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 964-968
- [36] S. Siemann, D. P. Evanoff, L. Marrone, A. J. Clarke, T. Viswanatha, G. I. Dmitrienko, *Antimicrob. Agents Chemother.* **2002**, *46*, 2450-2457
- [37] D. Minond, S. A. Saldanha, P. Subramaniam, M. Spaargaren, T. Spicer, J. R. Fotsing, T. Weide, V. V. Fokin, K. B. Sharpless, M. Galleni, C. Bebrone, P. Lassaux, P. Hodder, *Bioorg. Med. Chem.* **2009**, *17*, 5027-5037
- [38] T. Weide, S. A. Saldanha, D. Minond, T. P. Spicer, J. R. Fotsing, M. Spaargaren, J.-F. Frère, C. Bebrone, K. B. Sharpless, P. Hodder, V. V. Fokin, *ACS Med. Chem. Lett.* **2010**, *1*, 150-154
- [39] X. Wang, M. Lu, Y. Shi, Y. Ou, X. Cheng, *PLoSOne* **2015**, *10*, e0118290
- [40] M. Brindisi, S. Brogi, S. Giovani, S. Gemma, S. Lamponi, F. De Luca, E. Novellino, G. Campiani, J. D. Docquier, S. Butini, *J. Enz. Inhib. Med. Chem.* **2016**, *31(suppl1)*, 98-109
- [41] M. W. Walter, A. Felici, M. Galleni, R. P. Soto, R. M. Adlington, J. E. Baldwin, J.-F. Frère, M. Gololobov, C. J. Schofield, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2455-2458
- [42] D. J. Payne, J. A. Hueso-Rodríguez, H. Boyd, N. O. Concha, C. A. Janson, M. Gilpin, J. H. Bateson, C. Cheever, N. L. Niconovich, S. Pearson, S. Rittenhouse, D. Tew, E. Diez, P. Perez, J. De La Fuente, M. Rees, A. Rivera-Sagredo, *Antimicrob. Agents Chemother.* **2002**, *46*, 1880-1886
- [43] J. Brem, R. Cain, S. Cahill, M. A. McDonough, I. J. Clifton, J. C. Jimenez-Castellanos, M. B. Avison, J. Spencer, C. W. G. Fishwick, C. J. Schofield, *Nat. Comm.* **2016**, *7*:12406
- [44] J. Spencer, T. R. Walsh, *Angew. Chem. Int. Ed.* **2006**, *45*, 1022-1026; *Angew. Chem.* **2006**, *118*, 1038-1042
- [45] T. Christopheit, A. Albert, H.-K.S. Leiros, *Bioorg. Med Chem.* **2016**, *24*, 2947-2953
- [46] L. Nauton, R. Kahn, G. Garau, J.-F. Hernandez, O. Dideberg, *J. Mol. Biol.* **2008**, *375*, 257-269

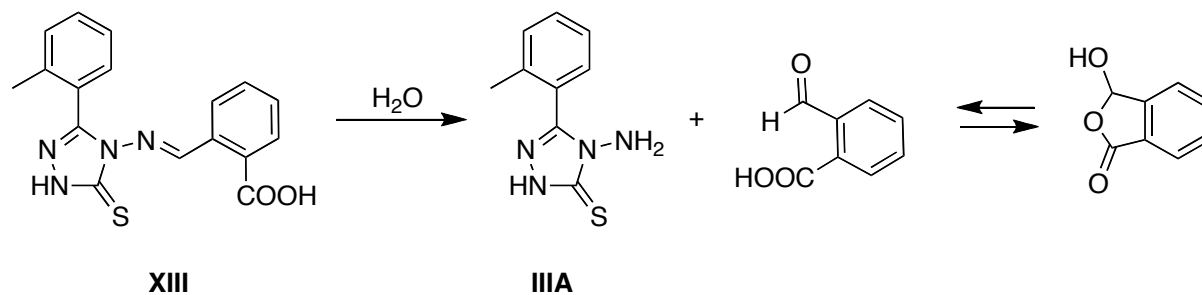
- [47] L. Olsen, S. Jost, H. W. Adolph, I. Pettersson, L. Hemmingsen, F. S. Jørgensen, *Bioorg. Med. Chem.* **2006**, *14*, 2627-2636
- [48] T. Christopeit, T. J. O. Carlsen, R. Helland, H. -K. S. Leiros, *J. Med. Chem.* **2015**, *58*, 8671-8682
- [49] N. F. Eweiss, A. A. Bahajaj, E. A. Elsherbini, *J. Heterocyclic Chem.* **1986**, *23*, 1451-1458
- [50] M. Ashok, B. S. Holla, B. Poojary, *Eur. J. Med. Chem.* **2007**, *42*, 1095-1101
- [51] B. Tozkoparan, N. Gökhan, G. Aktay, E. Yeşilada, M. Ertan, *Eur. J. Med. Chem.* **2000**, *35*, 743-750
- [52] A. T. Mavrova, D. Wesselinova, Y. A. Tsenov, P. Denkova, *Bioorg. Med. Chem.* **2009**, *44*, 63-69
- [53] Z. Li, Z. Gu, K. Yin, R. Zhang, Q. Deng, J. Xiang, *Eur. J. Med. Chem.* **2009**, *44*, 4716-4720
- [54] L. I. Kruse, C. Kaiser, W. E. DeWolf, J. A. Finkelstein, J. S. Frazee, E. L. Hilbert, S. T. Ross, K. E. Flaim, J. L. Sawyer, *J. Med. Chem.* **1990**, *33*, 781-789
- [55] J. L. Gilmore, B. W. King, N. Asakawa, K. Harrison, A. Tebben, J. E. 2nd Sheppeck, R. Q. Liu, M. Covington, J. J. Duan, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4678-4682
- [56] L. Maingot, F. Leroux, V. Landry, J. Dumont, H. Nagase, B. Villoutreix, O. Sperandio, R. Deprez-Poulain, B. Deprez, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6213-6216
- [57] Z. Amtul, M. Rasheed, M. I. Choudhary, R. Supino, K. M. Khan, Atta-Ur-Rahman, *Biochem. Biophys. Res. Commun.* **2004**, *319*, 1053-1063
- [58] P. Vella, W. M. Hussein, E. W. W. Leung, D. Clayton, D. L. Ollis, N. Mitic, G. Schenk, R. P. McGeary, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3282-3285
- [59] Faridoon, W. M. Hussein, P. Vella, N. U. Islam, D. L. Ollis, G. Schenk, R. P. McGeary, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 380-386
- [60] L. Feng, K. -W. Yang, L.-S. Zhou, J.-M. Xiao, X. Yang, L. Zhai, Y.-L. Zhang, M. W. Crowder, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 5185-5189
- [61] Y.-L. Zhang, K. W. Yang, Y. J. Zhou, A. E. LaCuran, P. Oelschlaeger, M. W. Crowder, *ChemMedChem* **2014**, *9*, 2445-2448
- [62] S.-K. Yang, J. S. Kang, P. Oelschlaeger, K. W. Yang, *ACS Med. Chem. Lett.* **2015**, *6*, 455-460
- [63] T. Christopeit, K. -W. Yang, S. -K. Yang, H. -K. S. Leiros, *Acta Cryst. F72*, **2016**, 813-819

- [64] N. O. Concha, C. A. Janson, P. Rowling, S. Pearson, C. A. Cheever, B. P. Clarke, C. Lewis, M. Galleni, J. -M. Frère, D. J. Payne, J. H. Bateson, S. S. Abdel-Meguid, *Biochemistry* **2000**, *39*, 4288-4298
- [65] I. Garcia-Saez, J.-D. Docquier, G. M. Rossolini, O. Dideberg, *J. Mol. Biol.* **2008**, *375*, 604-611
- [66] P. Lassaux, D. A. Traoré, E. Loisel, A. Favier, J.-D. Docquier, J. S. Sohler, C. Laurent, C. Bebrone, J.-M. Frère, J.-L. Ferrer, M. Galleni, *Antimicrob. Agents Chemother.* **2011**, *55*, 1248-1255
- [67] H. M. Zhang, Q. Hao, *FASEB J.* **2011**, *25*, 2574-2582
- [68] G. Garau, C. Bebrone, C. Anne, M. Galleni, J.-M. Frère, O. Dideberg, *J. Mol. Biol.* **2005**, *345*, 785-795
- [69] J. H. Ullah, T. R. Walsh, I. A. Taylor, D. C. Emery, C. S. Verma, S. J. Gamblin, J. Spencer, *J. Mol. Biol.* **1998**, *284*, 125-136
- [70] R. M. Breece, Z. Hu, B. Bennett, M. W. Crowder, D. L. Tierney, *J. Am. Chem. Soc.* **2009**, *131*, 11642-11643
- [71] a) Figure 1 was drawn using LigPlot: A. C. Wallace, R. A. Laskowski, J. M. Thornton, *Protein Eng.* **1996**, *8*, 127-134; b) Figures 2, 3 and 4 were drawn using UCSF Chimera software: E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, T. E. Ferrin, *J. Comput. Chem.* **2004**, *25*, 1605-1612
- [72] G. Turan-Zitouni, Z. A. Kaplancikli, K. Erol, F. S. Kiliç, *Il Farmaco* **1999**, *54*, 218-223
- [73] E. Cadag, E. Vitalis, K. P. Lennox, C. L. Ecale Zhou, A. T. Zemla, *BMC Res. Notes* **2012**, *5*:96
- [74] F. Baier, N. Tokuriki, *J. Mol. Biol.* **2014**, *426*, 2442-2456
- [75] J. H. Zhang, T. D. Chung, K. R. Oldenburg, *J. Biomol. Screen.* **1999**, *4*, 67-73
- [76] F. Sinturel, O. Pellegrini, S. Xiang, L. Tong, C. Condon, L. Bénard, *RNA* **2009**, *15*, 2057-2062
- [77] B. Kostecky, E. Pohl, A. Vogel, O. Schilling, W. Meyer-Klaucke, *J. Bacteriol.* **2006**, *188*, 1607-1614
- [78] A. Vogel, O. Schilling, M. Niecke, J. Bettmer, W. Meyer-Klaucke, *J. Biol. Chem.* **2002**, *277*, 29078-29085
- [79] M. Akoachere, R. Iozef, S. Rahlfs, M. Deponte, B. Mannervik, D. J. Creighton, R. H. Schirmer, K. Becker, *Biol. Chem.* **2005**, *386*, 41-52

Keywords: Metallo- β -Lactamase; 1,2,4-Triazole-3-thione; Bacterial resistance; β -Lactam antibiotic.

Accepted Manuscript

Scheme 1.



Scheme 2.

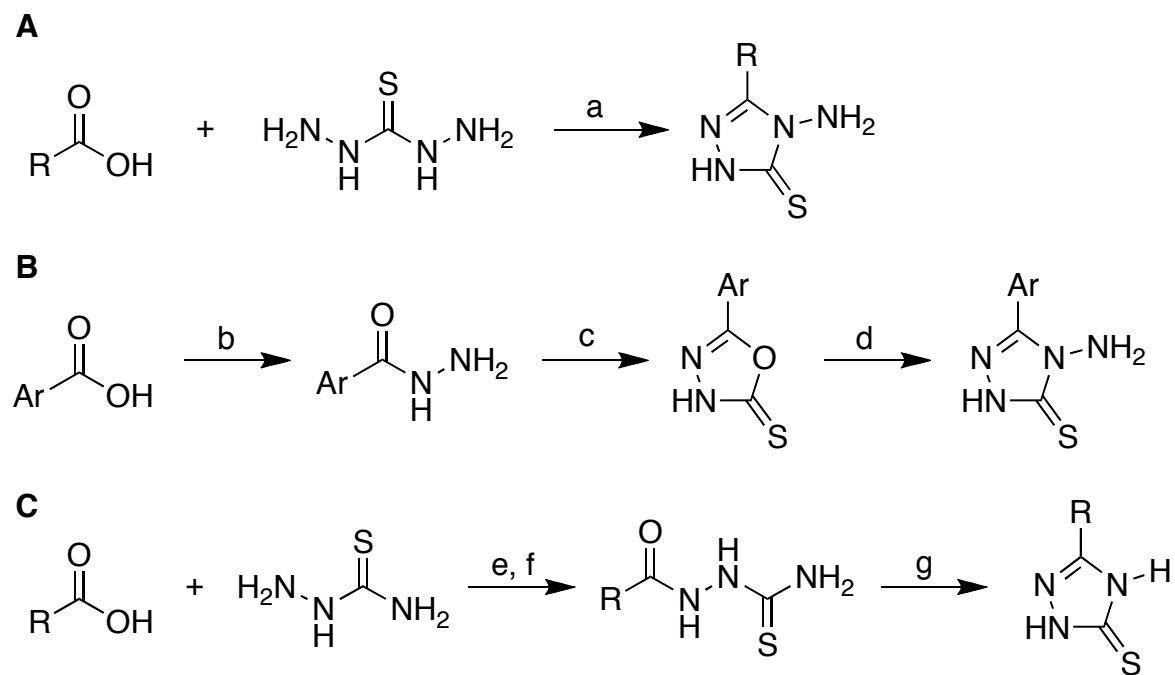


Figure 1.

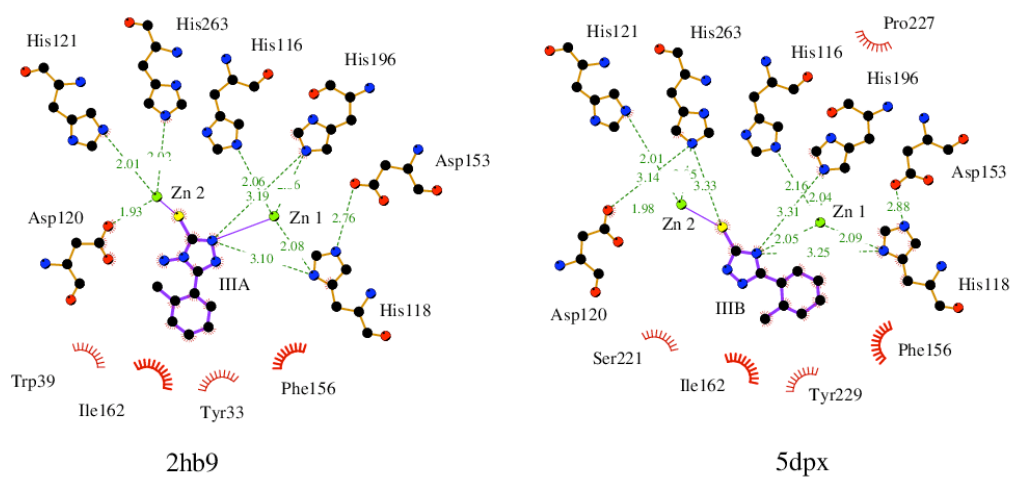


Figure 2.

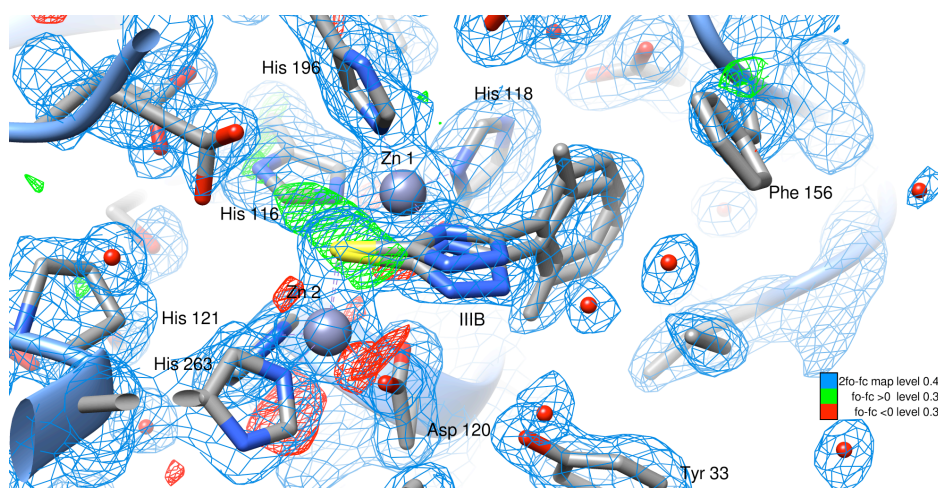


Figure 3.

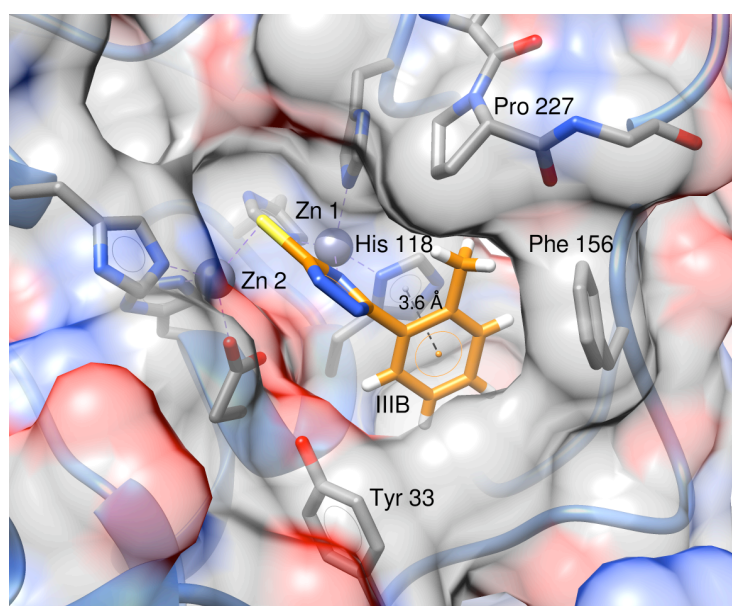


Figure 4A and 4B.

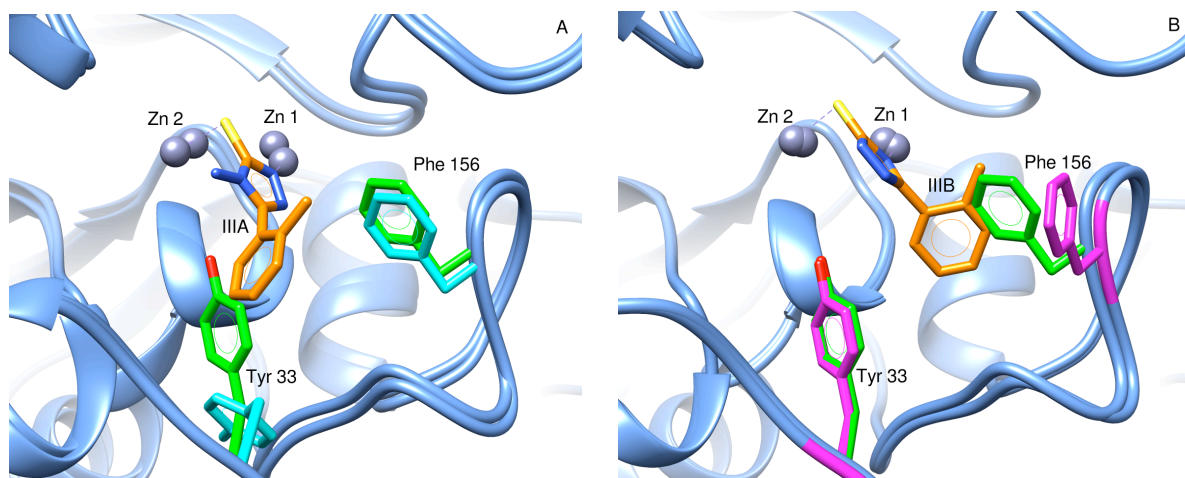


Figure 5.

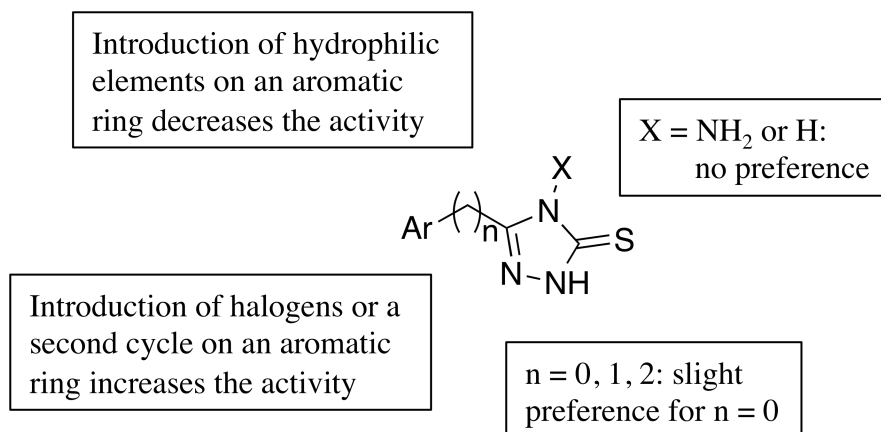
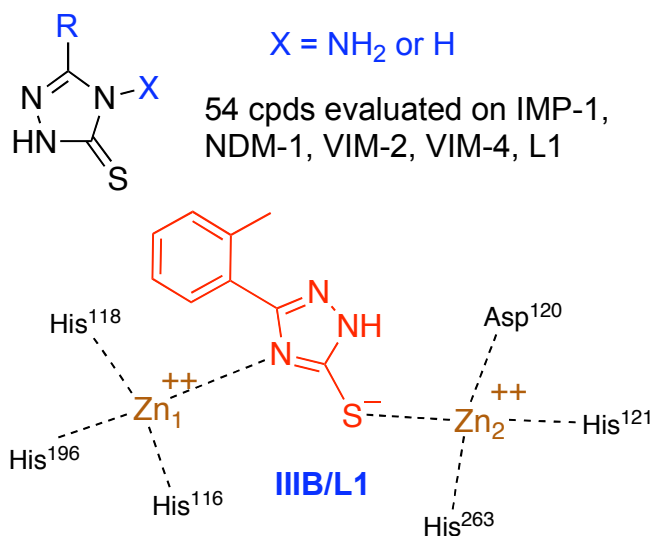


Table of Contents.

A series of **54 1,2,4-triazole-3-thione compounds** was synthesized and evaluated on a panel of metallo- β -lactamases involved in bacterial resistance. Some of them displayed IC₅₀ in the μ M range toward IMP-1, NDM-1 and VIM-2. Structural requirements for activity are discussed. The crystallographic structure of the complex L1/III B is reported and highlighted the original binding mode of this series. Finally, the activity on other metallo-enzymes belonging to the MBL fold superfamily was also evaluated.