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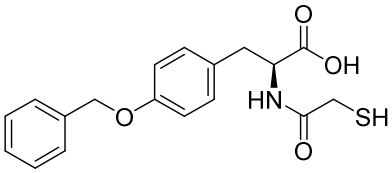
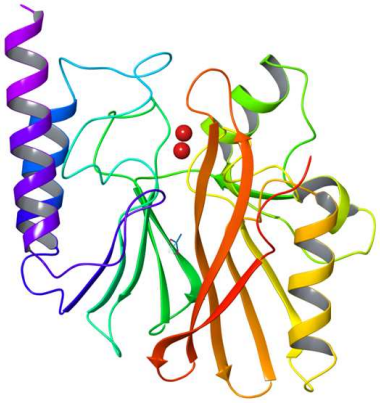
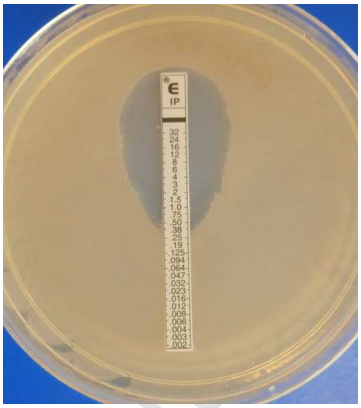


Competitive inhibitor of IMP-1
K_i = 86 nM





Design, synthesis, and *in vitro* and biological evaluation of potent amino acid-derived thiol inhibitors of the metallo-β-lactamase IMP-1

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Abstract

There are currently no clinically available inhibitors of metallo-β-lactamases (MBLs). These enzymes confer resistance to bacteria against a broad range of commonly used β-lactam antibiotics, and are produced by an increasing number of bacterial pathogens. In this study, several thiol derivatives of L-amino acids were designed and synthesized, and their inhibitory effects against the metallo-β-lactamase IMP-1 (subclass B1) were investigated. The most potent compound, derived from L-tyrosine, exhibited competitive inhibition, with a K_i of 86 nanomolar. The ability of this compound to render MBL-expressing bacteria susceptible to imipenem was examined. Reductions in MIC values up to 5.2—fold were observed.

1. Introduction

The β-lactam antibiotics are the most widely prescribed drugs for the treatment of bacterial infections, but in recent years the emergence of pathogenic bacteria that can secrete β-lactamases (enzymes that deactivate these antibiotics by hydrolyzing their β-lactam ring) has led to great concern regarding the continuing ability of current drugs to adequately treat diseases caused by these bacteria [1]. β-Lactamases may be classified as either serine-β-lactamases (SBLs, Classes A, C and D) or metallo-β-lactamases (MBLs, Class B), according to their mechanisms of action. MBLs are further divided into three subclasses, B1, B2 and B3, depending on their amino acid sequences and metal occupancies [2], and we have recently tentatively identified a fourth MBL subclass, B4 [3, 4]. There can be one or two zinc(II) ions in their active sites [3, 5-10].

Since MBLs can deactivate most β-lactam antibiotics, including such widely used drug families as the carbapenems, cephalosporins and penams [11, 12], the development of inhibitors of MBLs is an essential strategy for maintaining the usefulness of existing β-lactam antibiotics. While clavulanic acid is a potent inhibitor of SBLs [13], being widely prescribed in combination with amoxicillin, there are currently no clinically useful inhibitors against MBLs. Accordingly, the development of new MBL inhibitors is urgent [14, 15].

Various classes of MBL inhibitors have been reported [16], including trifluoromethyl ketones and alcohols [17], dicarboxylic acids [18-23], thiols [24-30], sulfates [31], hydroxamates [32], tetrazoles [33] and sulfonamides [34]. The aim of this work was to develop lead inhibitors of the MBL enzyme IMP-1 (subclass B1) through docking studies, synthesis, kinetic enzyme assays and cell-based assays. IMP-1, which may be secreted by both *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [35-38], was selected as our target since these pathogens have been responsible for outbreaks of antibiotic-

resistant bacterial infections in clinical settings. In addition, this enzyme is well-characterized and its crystal structure has been reported [39].

The design of this project was based on the work of Liénard *et al*. [40]. That group reported that the *N*-(3-mercapto)propanoyl derivative of unnatural D-phenylalanine was a competitive inhibitor of IMP-1, with a K_i of 0.088 micromolar (Fig. 1). The work presented here focusses on the design, synthesis and testing of thiol derivatives of the three natural aromatic amino acids, L-phenylalanine, L-tyrosine and L-tryptophan.

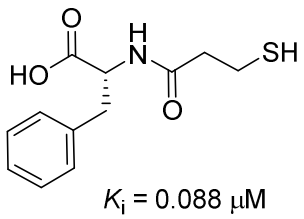
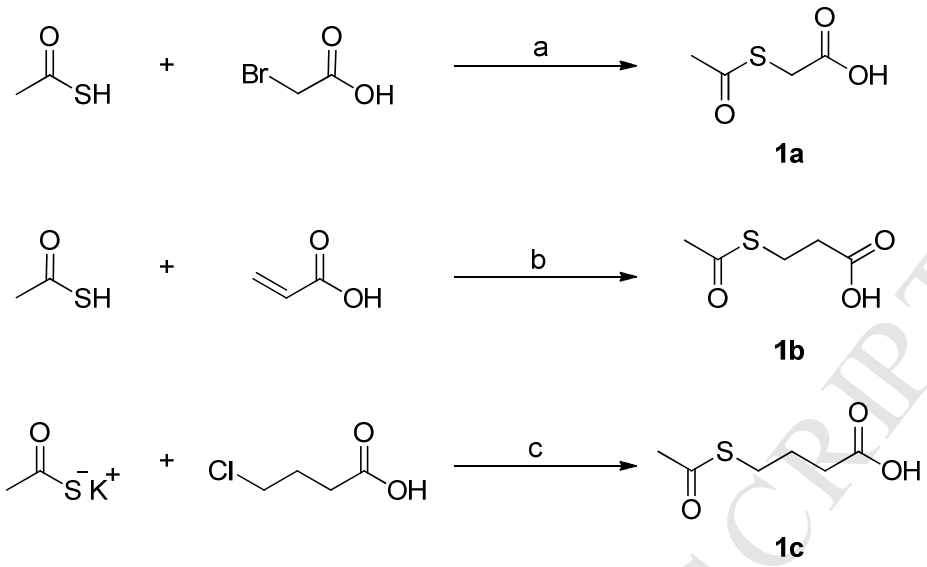


Figure 1. IMP-1 inhibitor reported by Liénard *et al*. [40].

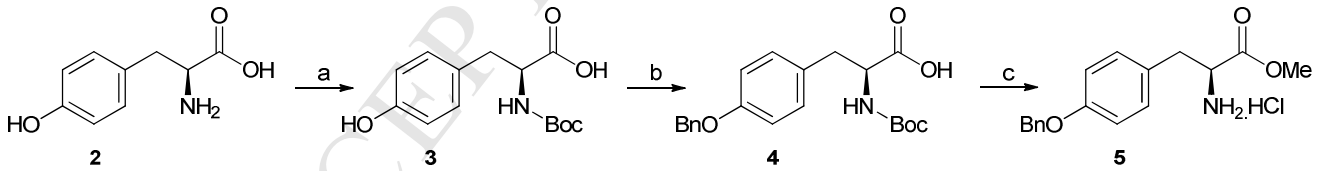
2. Chemistry

The synthetic routes to compounds **1a-c** are shown in Scheme 1. Compounds **1a** [41] and **1b** [42] were synthesized by the reaction of thioacetic acid with bromoacetic acid and acrylic acid, respectively. Compound **1c** [43] was prepared by the reaction of potassium thioacetate with 4-chlorobutanoic acid. In all cases isolated yields were around 60%.



Scheme 1. Reagents and conditions: (a) $NaHCO_3$, H_2O, r.t., overnight, 65%; (b) neat, 100 °C, 90 min, 62%; (c) DMF, r.t., 2 h, 59%.

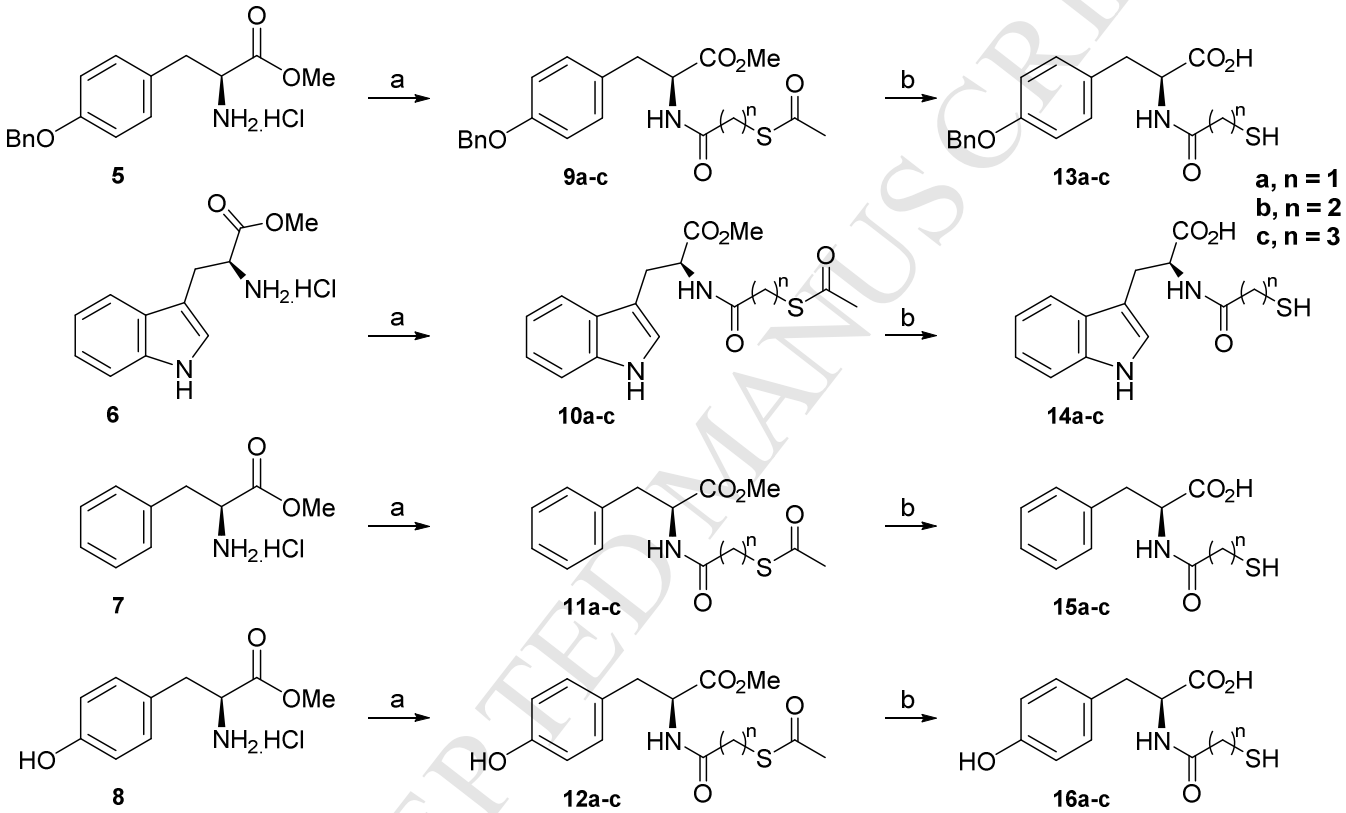
Boc-protected L-tyrosine **3** was synthesized from L-tyrosine **2** by standard methods [44]. Benzylation of the phenolic group of **3** using benzyl bromide and sodium methoxide in methanol produced benzyl ether **4** in 64% yield (Scheme 2) [45]. Heating benzyl ether **4** in refluxing methanol containing thionyl chloride produced the methyl ester hydrochloride salt **5** in quantitative yield.



Scheme 2. Reagents and conditions: (a) Boc_2O, NaOH, THF/H_2O, r.t., overnight, 100%; (b) BnBr, NaOMe, MeOH, 40 °C, 3 h, 64%; (c) $SOCl_2$, MeOH, Δ, 3 h, 100%.

L-Amino acid methyl esters **6-8** were synthesized by refluxing the appropriate L-amino acids in methanol containing thionyl chloride. Compounds **9a-c**, **10a-c**, **11a-c** and **12a-c** were synthesized by condensing the L-amino acid methyl esters with thioester-acid intermediates **1a-c** using HBTU/DIPEA

as the coupling reagent (Scheme 3). Hydrolyzed compounds **13a-c**, **14a-c**, **15a-c** and **16a-c** were prepared by treatment of intermediates **9a-c**, **10a-c**, **11a-c** and **12a-c** with degassed sodium hydroxide solution to give the final thiol products. Thiols **13a-c**, **14a-c**, **15a-c** and **16a-c** are sensitive to aerial oxidation, readily forming disulfides, which were apparent as trace impurities in the mass spectra of all of these thiols, even after careful purification by flash column chromatography.



Scheme 3. Reagents and conditions: (a) **1a, 1b or 1c**, HBTU, DIPEA, THF, r.t., overnight; (b) NaOH, H_2O/MeOH, r.t., 1 h.

3. Docking Studies

For computational modeling, the crystal coordinates of the IMP-1 enzyme (PDB code 1JJT) [19] were downloaded from the Protein Data Bank (PDB) and used in all subsequent docking studies. The IMP-1 MBL is functionally a monomeric protein but crystallizes as a dimer with 221 amino acids in each chain. Both chain A and chain B have identical amino acid residues and each has two zinc (II) ions in the active site. The topologies of the active sites of both chain A and chain B are identical and hence chain B was deleted from the crystal coordinates and only chain A was used for the docking studies. Since the enzyme kinetic assays (see Supplementary data) were performed at pH 7, the carboxylic acid functional groups of the docked inhibitors were deprotonated and anionic. As the thiol groups of the inhibitors were anticipated to coordinate to the zinc metal ions in the active site, these functional groups were also deprotonated for the docking studies. MolDock was used for all docking studies [46]. Calculated binding energies for all of the inhibitors (MolDock Scores) may be found in the Supplementary data.

Docking studies predicted that all of the inhibitors coordinated to both zinc ions in the active site of IMP-1 via their thiolate groups, with Zn—S distances of around 2.0—3.0 Ångstroms. Figure 2 shows the lowest energy docking result for the most potent inhibitor in the series, **13a** (see Section 4). In this example, Zn—S distances are less than 2.0 Å. A hydrogen bond between the amide carbonyl oxygen atom of **13a** and the side chain amide NH_2 group of Asn233 is present (3.2 Å). Distances between the central aromatic ring of **13a** and the aromatic rings of Trp64 and His263 are approximately 4 Å, slightly longer than the sum of the van der Waals radii of these groups.

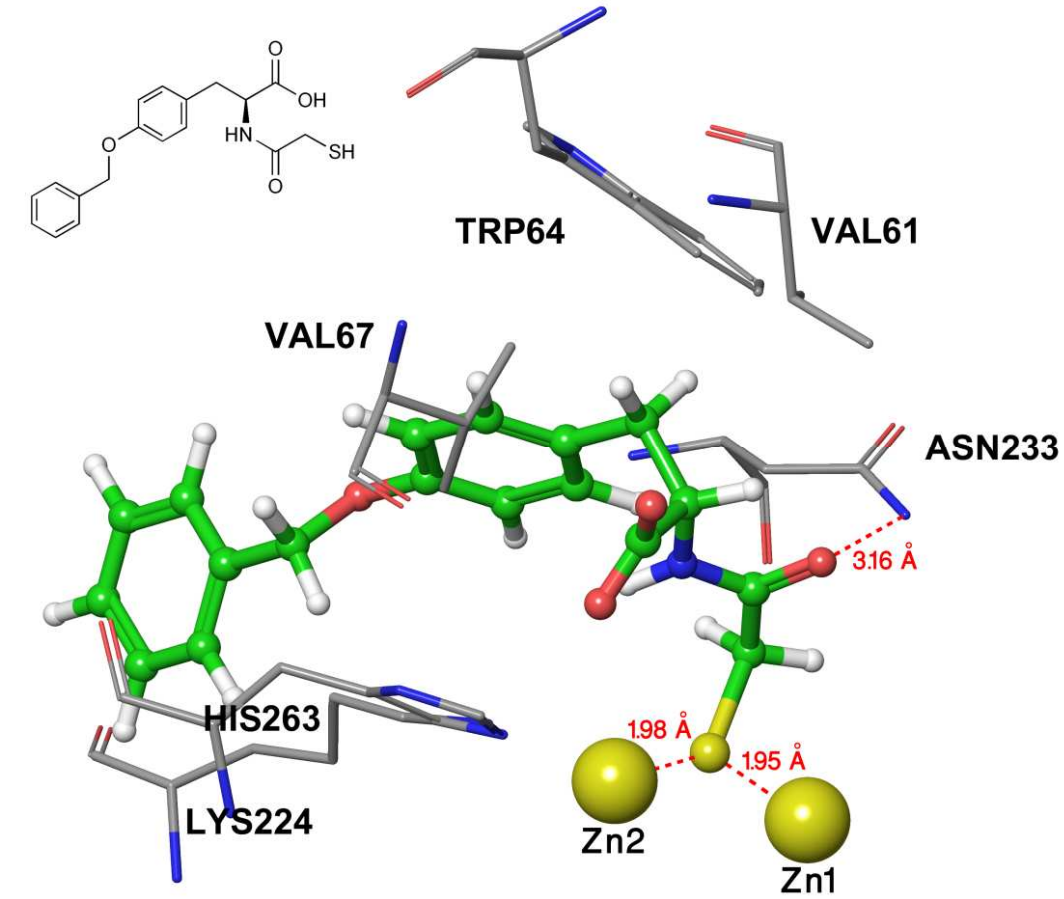


Figure 2: Interactions of docked ligand **13a** at the active site of IMP-1 (PDB: 1JJT). Atom colors are as follows: blue–nitrogen, red–oxygen, grey–carbon and yellow-zinc atom (of IMP-1), and red–oxygen, blue–nitrogen, green–carbon, and yellow–sulfur (on inhibitor).

4. Enzyme Kinetics Studies

The inhibitory activities (IC_{50} values) of the inhibitors against IMP-1 were first evaluated by a screening assay which measured the residual activity of the enzyme. In our previous work in the area of MBL inhibitors [28-30], we have employed the chromogenic cephalosporin CENTA [47] as substrate in our enzyme assays. However, thiol-containing inhibitors react with this compound, displacing the 5-nitro-2-

sulfidobenzoate chromophore [38]. Therefore, in this study benzylpenicillin was used as the substrate. Initial assay results showed that all of the thiol compounds exhibited more than 50% inhibition at 10 μM. Further kinetic analyses then allowed the detemination of the competitive (K_{ic}) and uncompetitive (K_{iuc}) values of these compounds (Table 1). A representative inhibition curve of compound **13a** is shown in the Supplementary data.

Table 1

Inhibitory activities of the target compounds **13a-c**, **14a-c**, **15a-c**, **16a-c** and **1a** against IMP-1. Benzylpenicillin was used as the substrate.

Compound	K_{ic} (μM)[a]	K_{iuc} (μM)[b]
13a	0.086 ± 0.024	-[c]
13b	9.39 ± 4.60	-
13c	5.85 ± 1.30	-
14a	0.47 ± 0.09	-
14b	5.02 ± 1.64	-
14c	8.62 ± 3.30	-
15a	0.25 ± 0.20	-
15b	1.45 ± 0.67	60.25 ± 20
15c	2.82 ± 1.98	-

16a	4.55 ± 1.96	-
16b	5.18 ± 1.55	-
16c	5.63 ± 4.50	-
1a	0.32 ± 4.50	2.64 ± 0.46

[a] K_{ic}: Inhibition constant for competitive inhibition mode.

[b] K_{iuc}: Inhibition constant for uncompetitive inhibition mode.

[c] - : Insignificant (> 1 mM) contribution of this mode.

All of the compounds demonstrated K_{ic} values lower than 10 µM against IMP-1. It was observed that the shorter-chain thioacetic acid-derived inhibitors (**13a**, **14a** and **15a**) were more potent than their longer chain counterparts (Table 1). In most cases the inhibitory mode was exclusively competitive; the exception was compound **15b** which showed a mixed mode of inhibition, although its K_{iuc} value (60 µM) was much larger than its K_{ic} value (1.45 µM). Compound **13a** exhibited the most potent inhibitory activity with a K_{ic} value of 86 nM. Since several low-molecular weight thiols have been reported to exhibit moderate inhibitory activities against MBLs [24-26], we also assayed the thioacetate intermediates **1a-c**. Interestingly, compound **1a** was a mixed-mode inhibitor of IMP-1 with a K_{ic} value of 0.32 µM. Compounds **1b** and **1c** showed no inhibitory activity towards IMP-1 (data not shown).

5. Biological Evaluation

The effects of the most potent IMP-1 inhibitor, compound **13a**, were examined on the three following MBL-producing bacterial strains: 1) *Escherichia coli* expressing IMP-1 (*i.e.* bla_{IMP-1} expressed in BL21 cells); 2) a clinical isolate of *Enterobacter cloacae* expressing the very closely related MBL IMP-4 (95% homology) [48]; and 3) a clinical isolate of *E. cloacae* expressing both IMP-4 and TEM-1, a Class A serine-β-lactamase [2]. The imipenem MIC in combination with **13a** was tested by incorporating 20 μL of **13a** at a concentration of 50 mM into Mueller-Hinton (MH) agar. Bacterial suspensions at 0.5 McFarland were spread onto the agar plates supplemented with compound **13a**. The imipenem E-test strip (bioMerieux) was then placed on the media. As a control the imipenem MICs of the three test strains were also determined on plain MH agar (*i.e.* in the absence of **13a**).

Table 2 summarizes the results of these trials. Administration of **13a** to the *E. cloacae* strain expressing both IMP-4 and TEM-1 resulted in no difference in the growth of the bacteria when imipenem was administered. For the *E. cloacae* expressing only IMP-4, a marked reduction in the imipenem MIC (3.9–fold) was noted (Fig. 3). For *E. coli* expressing only IMP-1, an even greater reduction in the imipenem MIC was observed (5.2–fold) (Fig. 4).

Table 2

Imipenem MIC values in the absence or presence of compound **13a**

Strains	β-Lactamases	Imipenem MICs (μg/mL)		Reduction in MIC
		Without inhibitor	With **13a**	
E. coli BL21	IMP-1	2	0.38	5.2—fold
E. cloacae ECL1	IMP-4	1.5	0.38	3.9—fold

E. cloacae ECL2	IMP-4, TEM-1	0.5	0.5	None

The results suggest that **13a**, as well as being a potent *in vitro* inhibitor of IMP-1, also significantly inhibits IMP-1 and IMP-4 in strains of *E. coli* and *E. cloacae* that express this MBL and its variant. The absence of an effect of **13a** on the strain of *E. cloacae* that expresses the serine-β-lactamase TEM-1 is expected, as **13a** would not be anticipated to inhibit this enzyme, and hence would have no effect on the ability of TEM-1 to degrade imipenem.

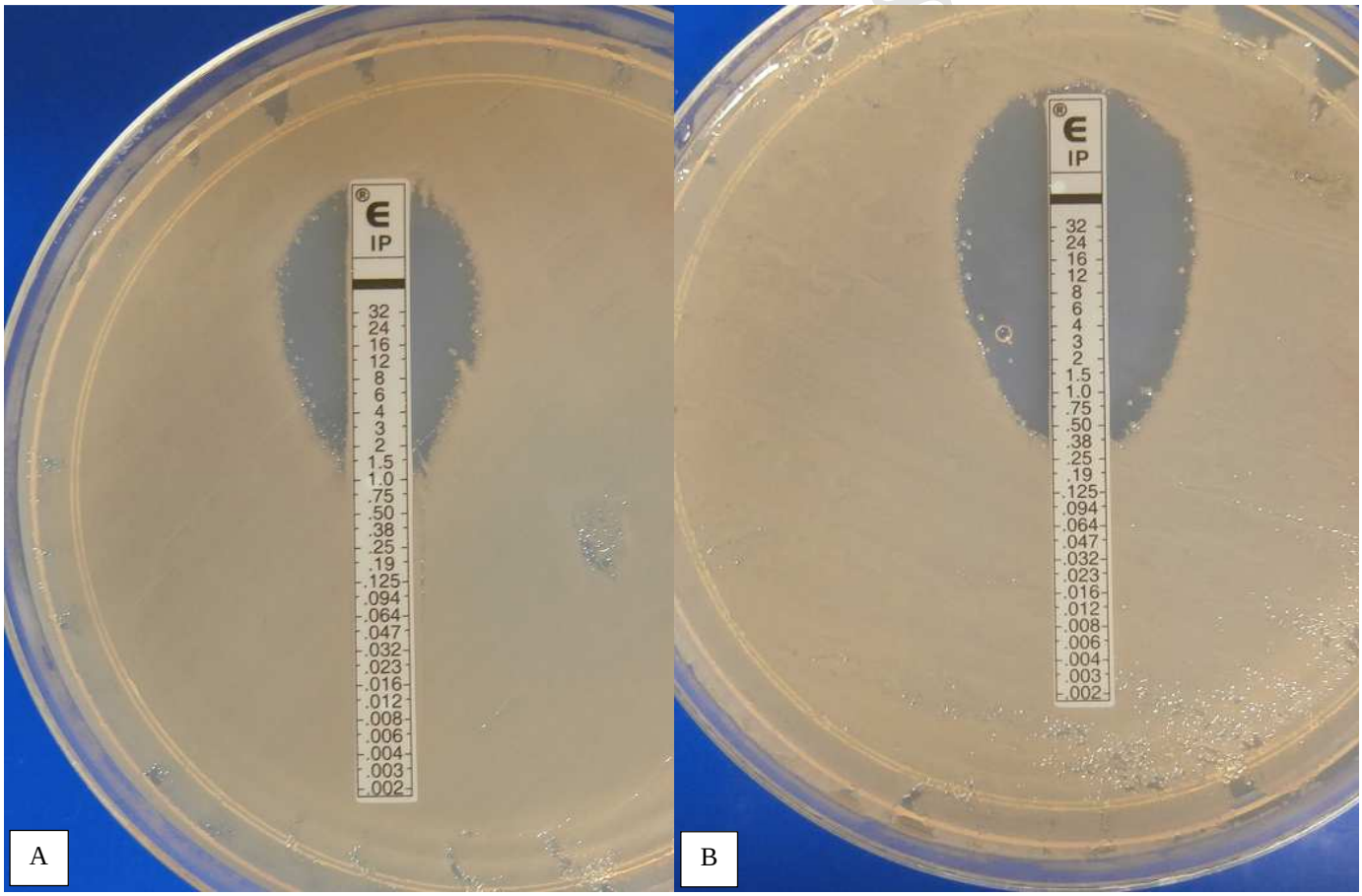


Figure 3. Imipenem-induced growth inhibition of *E. cloacae* ECL1 expressing IMP-4 in the absence of an MBL inhibitor (A, left) and the presence of compound **13a** (B, right).

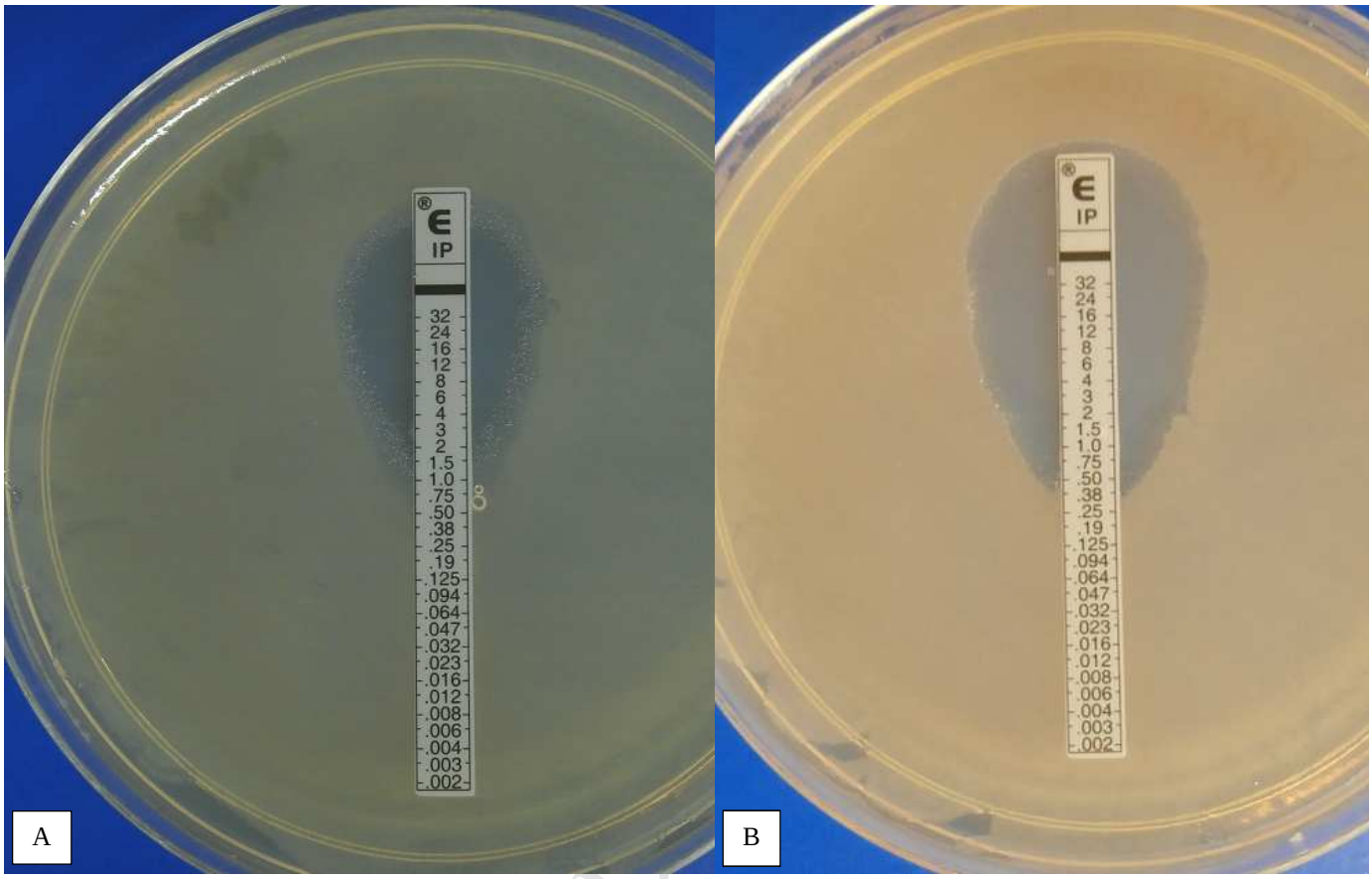


Figure 4. Imipenem-induced growth inhibition of *E. coli* BL21 expressing IMP-1 in the absence of an MBL inhibitor (A, left) and the presence of compound **13a** (B, right).

6. Conclusions

We describe a straightforward and efficient synthesis of novel thiol compounds based on naturally occurring aromatic amino acids as inhibitors against the metallo-β-lactamase IMP-1. All of these compounds demonstrated promising *in vitro* inhibitory activity against IMP-1. Among them, compound **13a** is the most potent inhibitor, with a competitive K_i of 86 nanomolar. Importantly, this inhibitor also

significantly decreases the imipenem MIC for bacteria expressing IMP-1 or IMP-4. We expect that this compound can be used as a lead for further development of potent MBL inhibitors of clinical relevance.

7. Experimental

All chemicals were purchased from Sigma-Aldrich, Merck and Fluka chemical companies. All NMR experiments were recorded on Bruker AVANCE 500, 400 or 300 MHz spectrometers. Chemical shifts are reported in parts per million (ppm) on a δ scale and referenced to the residual solvent peak (^{1}H, 7.24 ppm, ^{13}C, 77.0 ppm for $CDCl_3$; ^{1}H, 2.49 ppm, ^{13}C, 39.5 ppm for DMSO-d_6; ^{1}H, 3.30 ppm, ^{13}C, 49.5 ppm for CD_3OD). Coupling constants (*J*) are reported in Hertz. Multiplicities of the peaks are abbreviated as follows: s for singlet, bs for broad singlet, d for doublet, t for triplet, and q for quartet. Low- and high-resolution EI-MS were measured on a Finnigan MAT 900 XL-Trap mass spectrometer in positive and negative ionization mode. LR-ESI were recorded on a Bruker HCT 3D Ion Trap and HR-ESI were performed on a BrukerMicrOTof-Q with the DIONEX Ultimate 3000 LC in positive and negative electrospray ionization mode, with CH_3OH as solvent. For IR analyses samples were measured neat on a PerkinElmer Frontier FT-IR Spectrometer.

7.1. Enzyme expression

The IMP-1 enzyme, lacking the first 21 signal peptide amino acid residues, was expressed and purified using the protocol of Vella *et al.* [38, 49]. Details of the enzyme kinetic assays are presented in the Supplementary data.

7.2. General procedure 1: esterification of amino acids

Compounds **6-8** were synthesized using the following general procedure reported by Maiti and Banerjee [50]: a solution of amino acid (18.2 mmol) in MeOH (20 mL) was stirred at 0 °C. Thionyl chloride (1.6

mL, 22 mmol) was added dropwise over 10 minutes. The reaction mixture was then allowed to warm to room temperature before being heated under reflux for 3 hours. The solvent and volatiles were evaporated under reduced pressure and the product was triturated with ethyl acetate to give the methyl ester hydrochloride salt as a colorless solid (100%).

7.3. General procedure 2: amide coupling reactions

Compounds **9a-c**, **10a-c, 11a-c** and **12a-c** were synthesized using the following general procedure: L-amino acid methyl ester hydrochlorides **5-8** (4.50 mmol), thioesters **1a-c** (4.95 mmol), HBTU (1.88 g, 4.95 mmol), DIPEA (1.75 g, 2.35 mL, 13.5 mmol) in THF (40 mL) were stirred overnight. The THF was evaporated under reduced pressure then either DCM or ethyl acetate (40 mL) was added. The organic solution was washed with saturated sodium bicarbonate solution (40 mL), dried (Na_2SO_4), filtered and evaporated to give the crude product, which was purified by flash chromatography (30-100% EtOAc in petroleum ether).

7.4. General procedure 3: saponification

Compounds **13a-c**, **14a-c, 15a-c** and **16a-c** were synthesized using the following general procedure: Compounds **9a-c**, **10a-c, 11a-c** and **12a-c** (2.4 mmol) were dissolved in degassed methanol (10 mL), then degassed sodium hydroxide solution (1 M, 10 mL) was added and the solution was stirred under argon for one hour. The organic solvent was evaporated under reduced pressure, then degassed water (30 mL) was added and the water phase was washed with degassed ethyl acetate (40 mL). The pH was adjusted to 1 using concentrated HCl. The solution was extracted with ethyl acetate (40 mL) and washed with water (5×50 mL). The organic phase was dried (Na_2SO_4), filtered and evaporated under reduced pressure to give the pure products.

*7.5. 2-(Acetylthio)acetic acid (**1a**)*

A variation of the method of Benary was used [41]. Sodium bicarbonate (15.7 g, 187 mmol) was added slowly to a stirred solution of thioacetic acid (8.1 g, 7.5 mL, 106 mmol) and bromoacetic acid (11.2 g, 80 mmol) in water (100 mL). The mixture was stirred at room temperature overnight, then acidified to pH 1 with concentrated HCl and extracted with ethyl acetate (100 mL). The organic layer was separated and washed with water (3×100 mL), then dried (Na_2SO_4), filtered and evaporated to give 2-(acetylthio)acetic acid (**1a)** as a yellow oil (7.05 g, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.44 (1H, s, OH), 3.71 (2H, s, CH_2), 2.37 (3H, s, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.9 (SCO), 174.5 (CO_2), 31.2 (CH_2), 30.0 (CH_3). The NMR spectra are in agreement with the literature [51].

*7.6. 3-(Acetylthio)propanoic acid (**1b**)*

A variation on the method of Li *et al*. was used [42]. Thioacetic acid (10.8 g, 10 mL, 142 mmol) was added with stirring to acrylic acid (7.9 g, 7.5 mL, 110 mmol). The mixture was stirred under argon for 30 min at room temperature and then at 100 °C for 90 min. Crystals formed on cooling to room temperature. Excess thioacetic acid was removed under reduced pressure then the residue was dissolved in ethyl acetate (100 mL). The solution was washed with water (3×100 mL) then dried (Na_2SO_4), filtered and evaporated to give 3-(acetylthio)propanoic acid (**1b**) as a yellow hygroscopic solid (10.1 g, 62%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.01 (1H, bs, OH), 3.08 (2H, t, *J*=6.9 Hz, SCH_2), 2.67 (2H, t, *J*=6.9 Hz, CH_2CO), 2.31 (3H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.5 (SCO), 177.7 (CO_2), 34.1 (CH_2S), 30.5 ($\underline{C}H_2CO$), 23.8 (CH_3). The ^{1}H NMR spectrum is in agreement with the literature [52].

*7.7. 4-(Acetylthio)butanoic acid (**1c**)*

The method of Hogg *et al.* was adapted [43]. Potassium thioacetate (1.0 g, 8.8 mmol) was added portionwise to a solution of 4-chlorobutyric acid (1.0 g, 8.2 mmol) in dry DMF (5 mL) at room temperature. The reaction mixture was stirred under argon for 2 hours then the organic solvent was removed under reduced pressure. The residue was taken up in ethyl acetate (50 mL) and washed with HCl (5%, 2×50 mL) and water (3×50 mL). The organic phase was dried (Na_2SO_4), filtered and evaporated to give 4-(acetylthio)butanoic acid (**1c**) as a dark oil (0.78 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.91 (2H, t, *J*=7.1 Hz, CH_2S), 2.42 (2H, t, *J*=7.4 Hz, $C\underline{H}_2CO$), 2.32 (3H, s, CH_3), 1.91 (2H, quintet, *J*=7.2 Hz, $CH_2C\underline{H}_2CH_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.6 (SCO), 178.3 (CO_2), 32.5 ($\underline{C}H_2CO$), 30.6 (CH_2S), 28.2 (CH_3), 24.5 ($CH_2\underline{C}H_2CH_2$). The NMR spectra are in agreement with the literature [43].

*7.8. (S)-Methyl 2-amino-3-(4-(benzyloxy)phenyl)propanoate hydrochloride (**5**)*

White solid, 5.85 g (100%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.63 (3H, bs, NH_3), 7.47-7.25 (5H, m, Ar-H), 7.14 (2H, d, *J*=8.5 Hz, Ar-H), 6.96 (2H, d, *J*=8.5 Hz, Ar-H), 5.07 (2H, s, $PhC\underline{H}_2O$), 4.18 (1H, t, *J*=6.9 Hz, $C\underline{H}NH_3$), 3.65 (3H, s, OCH_3), 3.09-3.00 (2H, m, $C\underline{H}_2CHN$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 169.8 (CO_2), 158.0 (Ar-C), 137.5 (Ar-C), 131.0 (Ar-C), 128.8 (Ar-C), 128.2 (Ar-C), 128.1 (Ar-C), 127.0 (Ar-C), 115.3 (Ar-C), 69.6 (CH_2O), 53.7 (CHN), 53.0 (OCH_3), 35.4 ($\underline{C}H_2CHN$). The NMR spectra are in agreement with the literature [53].

*7.9. (S)-Methyl 2-(2-(acetylthio)acetamido)-3-(4-(benzyloxy)phenyl)propanoate (**9a**)*

Yellow-brown oil, 1.63 g (90%). IR ν_{max}: 3676, 3269, 2988, 2901, 1733 (CO_2), 1700 (COS) 1656 (CON), 1511, 733 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.45-7.26 (5H, m, Ar-H), 6.98 (2H, d, *J*=8.5 Hz, Ar-H), 6.87 (2H, d, *J*=8.5 Hz, Ar-H), 6.58 (1H, d, *J*=7.8 Hz, NH), 5.02 (2H, s, PhC$\underline{H}_2$O), 4.76 (1H, dt, *J*=7.8, 5.8 Hz, C$\underline{H}$NH), 3.71 (3H, s, OCH_3), 3.55 (1H, d, *J*=15.0 Hz, C$\underline{H}_A$$H_B$S), 3.49 (1H, d, *J*=15.0 Hz, $CH_A\underline{H}_B$S), 3.08 (1H, dd, *J*=14.1, 5.7 Hz, C$\underline{H}_A$$H_B$CHN), 3.00 (1H, dd, *J*=14.1, 6.7 Hz, $CH_AC\underline{H}_B$CHN), 2.31 (3H, s, CH_3CO). ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.1 (COS), 171.5 (CO_2), 167.5 (CON), 157.8 (Ar-C), 136.9 (Ar-C), 130.2 (Ar-C), 128.5 (Ar-C), 127.9 (Ar-C), 127.8 (Ar-C), 127.4 (Ar-C), 114.8 (Ar-C), 69.9 (Ph$\underline{C}H_2$O), 53.5 ($\underline{C}$HN), 52.3 (OCH_3), 36.7 ($\underline{C}H_2$CHN), 32.7 (CH_2S), 30.1 ($\underline{C}H_3$CO). HRMS calculated for $C_{21}H_{23}NNaO_5S$ $[M+Na]^+$ 424.1189, found 424.1202.

*7.10. (S)-3-(4-(Benzyloxy)phenyl)-2-(2-mercaptoacetamido)propanoic acid (**13a**)*

Colorless solid, 0.70 g (84%). IR ν_{max}: 3676, 3325, 2989, 2598 (SH), 2901, 1702 (CO_2), 1610 (CON), 1543, 1512, 1235, 736 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 8.22 (1H, d, *J*=8.2 Hz, NH), 7.40-7.27 (5H, m, Ar-H), 7.09 (2H, d, *J*=9.2 Hz, Ar-H), 6.87 (2H, d, *J*=9.2 Hz, Ar-H), 5.01 (2H, s, PhC$\underline{H}_2$O), 4.31 (1H, dt, *J*=7.8, 5.8 Hz, C$\underline{H}$NH), 3.06 (2H, s, CH_2S), 2.93 (1H, dd, *J*=13.7, 5.0 Hz, C$\underline{H}_A$$H_B$CHN), 2.77 (1H, dd, *J*=13.7, 6.8 Hz, $CH_A\underline{H}_B$CHN). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.7 (CO_2), 169.4 (CON), 157.0 (Ar-C), 137.2 (Ar-C), 130.2 (Ar-C), 129.4 (Ar-C), 128.4 (Ar-C), 127.7 (Ar-C), 127.6 (Ar-C), 114.5 (Ar-C), 69.1 (CH_2O), 53.8 (CHN), 35.9 ($\underline{C}H_2$CHN), 26.8 (CH_2S). HRMS calculated for $C_{18}H_{18}NO_4S$ $[M-H]^-$ 344.0962, found 344.0949.

*7.11. (S)-Methyl 2-(3-(acetylthio)propanamido)-3-(4-(benzyloxy)phenyl)propanoate (**9b**)*

Pale brown oil, 1.50 g (80%). IR ν_{max}: 3675, 3304, 2989, 2901, 1740 (CO_2), 1679 (COS), 1650 (CON), 1510, 1233, 1109, 737 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.42-7.25 (5H, m, Ar-H), 7.00 (2H, d, *J*=8.5 Hz, Ar-H), 6.87 (2H, d, *J*=8.5 Hz, Ar-H), 6.04 (1H, d, *J*=7.7 Hz, NH), 5.01 (2H, s, PhC$\underline{H}_2$O),

4.81 (1H, dt, *J*=7.8, 5.8 Hz, C<u>H</u>NH), 3.69 (3H, s, CH_3O), 3.13-2.96 (4H, m, S<u>CH</u>$_2$ and <u>CH</u>$_2$CHN), 2.49 (2H, dt, *J*=6.8, 3.0 Hz, <u>CH</u>$_2$CO), 2.28 (3H, s, CH_3CO). ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.9 (COS), 172.0 (CON), 170.2 (CO_2), 158.0 (Ar-C), 136.9 (Ar-C), 130.3 (Ar-C), 128.6 (Ar-C), 127.98 (Ar-C), 127.97 (Ar-C), 127.5 (Ar-C), 114.9 (Ar-C), 70.0 (CH_2O), 53.3 (CHNH), 52.3 (OCH_3), 37.0 (<u>C</u>H$_2$CHN), 36.0 (CH_2S), 30.6 (<u>C</u>H$_2$CO), 24.7 (<u>C</u>H$_3$CO). HRMS calculated for $C_{22}H_{25}NNaO_5S$ $[M+Na]^+$ 438.1346, found 438.1352.

*7.12. (*S*)-3-(4-(Benzyloxy)phenyl)-2-(3-mercaptopropanamido)propanoic acid (**13b**)*

Pale yellow viscous oil, 0.71 g (82%). IR ν_{max}: 3676, 3325, 2989, 2598 (SH), 2901, 1702 (CO_2), 1610 (CON), 1543, 1511, 1232, 736 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD): δ 7.41-7.21 (5H, m, Ar-H), 7.13 (2H, d, *J*=9.2 Hz, Ar-H), 6.84 (2H, d, *J*=9.2 Hz, Ar-H), 4.97 (2H, s, PhC<u>H</u>$_2$O), 4.51 (1H, dd, *J*=7.5, 5.5 Hz, C<u>H</u>NH), 3.14 (1H, dd, *J*=14.0, 5.0 Hz, C<u>H</u>$_A$H$_B$CHN), 2.88 (1H, dd, *J*=14.0, 7.9 Hz, CH$_A$<u>H</u>$_B$CHN), 2.62 (2H, dt, *J*=6.5, 2.6 Hz, CH_2S), 2.40 (2H, dt, *J*=7.5, 2.5 Hz, CH_2CO). ^{13}C NMR (100 MHz, CD_3OD): δ 173.3 (CO_2), 172.8 (CON), 158.9 (Ar-C), 138.9 (Ar-C), 131.64 (Ar-C), 131.55 (Ar-C), 128.7 (Ar-C), 128.5 (Ar-C), 115.69 (Ar-C), 115.66 (Ar-C), 70.9 (CH_2O), 57.1 (CHN), 38.3 (<u>C</u>H$_2$CHN), 37.4 (<u>C</u>H$_2$CO), 28.3 (CH_2S). HRMS calculated for $C_{19}H_{20}NO_4S$ $[M-H]^-$ 358.1119, found 358.1110.

*7.13. (*S*)-Methyl 2-(4-(acetylthio)butanamido)-3-(4-(benzyloxy)phenyl)propanoate (**9c**)*

Brown viscous oil, 1.35 g (70%). IR ν_{max}: 3676, 3292, 2989, 2901, 1749, 1736 (CO_2), 1688 (COS), 1650 (CON), 1610, 1544, 1242, 697 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43-7.26 (5H, m, Ar-H), 6.99 (2H, dt, *J*=8.5, 2.7 Hz, Ar-H), 6.88 (2H, dt, *J*=8.5, 2.4 Hz, Ar-H), 6.03 (1H, d, *J*=7.8 Hz, NH), 5.01 (2H, s, PhC<u>H</u>$_2$O), 4.82 (1H, dt, *J*=7.8, 3.9 Hz, C<u>H</u>NH), 3.70 (3H, s, CH_3O), 3.08 (1H, dd, *J*=14.0, 5.8 Hz, C<u>H</u>$_A$H$_B$CHN), 2.99 (1H, dd, *J*=14.0, 6.1 Hz, CH$_A$<u>H</u>$_B$CHN), 2.83 (2H, dt, *J*=7.1, 4.1 Hz, CH_2S), 2.30 (3H, s, CH_3CO), 2.22 (2H, dt, *J*=5.4, 1.7 Hz, CH_2CO), 1.86 (2H, quintet, *J*=7.2 Hz, CH_2C<u>H</u>$_2CH_2$). ^{13}C

NMR (100 MHz, $CDCl_3$): δ 195.9 (COS), 172.2 (CON), 171.5 (CO_2), 157.9 (Ar-C), 136.9 (Ar-C), 130.2 (Ar-C), 128.5 (Ar-C), 128.1 (Ar-C), 127.9 (Ar-C), 127.4 (Ar-C), 114.9 (Ar-C), 70.0 (CH_2O), 53.1 (CHN), 52.3 (OCH_3), 37.0 ($\underline{C}H_2CHN$), 34.8 ($\underline{C}H_2CO$), 30.6 (CH_2S), 28.3 ($\underline{C}H_3CO$), 25.4 ($CH_2\underline{C}H_2CH_2$). HRMS calculated for $C_{23}H_{27}NNaO_5S$ $[M+Na]^+$ 452.1502, found 452.1511.

*7.14. (S)-3-(4-(Benzyloxy)phenyl)-2-(4-mercaptobutanamido)propanoic acid (**13c**)*

Brown semisolid, 0.72 g (80%). IR ν_{max}: 3675, 3325, 2989, 2598 (SH), 2925, 1722 (CO_2), 1611 (CON), 1510, 1226, 1176, 695 cm^{-1}. ^1H NMR (500 MHz, CD_3OD): δ 7.40 (2H, t, *J*=7.9 Hz, Ar-H), 7.35 (2H, t, *J*=7.1 Hz, Ar-H), 7.28 (1H, t, *J*=6.5 Hz, Ar-H), 7.12 (2H, d, *J*=7.8 Hz, Ar-H), 6.90 (2H, d, *J*=8.8 Hz, Ar-H), 5.04 (2H, s, $PhC\underline{H}_2O$), 4.64 (1H, dd, *J*=7.2, 4.8 Hz, $C\underline{H}NH$), 3.21 (1H, dd, *J*=14.0, 4.4 Hz, $C\underline{H}_AH_BCHN$), 2.85 (1H, dd, *J*=14.0, 9.8 Hz, $CH_A\underline{H}_BCHN$), 2.31 (2H, t, *J*=7.0 Hz, CH_2CO), 2.28-2.21 (2H, m, CH_2S), 1.79-1.70 (2H, m, $CH_2C\underline{H}_2CH_2$). ^{13}C NMR (125 MHz, CD_3OD): δ 175.2 (CO_2), 174.9 (CON), 159.2 (Ar-C), 138.8 (Ar-C), 131.3 (Ar-C), 130.8 (Ar-C), 129.5 (Ar-C), 128.8 (Ar-C), 128.6 (Ar-C), 115.9 (Ar-C), 71.0 (CH_2O), 55.0 (CHN), 37.6 ($\underline{C}H_2CHN$), 35.2 ($\underline{C}H_2CO$), 31.2 (CH_2S), 24.2 ($CH_2\underline{C}H_2CH_2$). HRMS calculated for $C_{20}H_{23}NO_4S$ $[M-H]^-$ 372.1275, found 372.1269.

*7.15. (S)-Methyl 2-amino-3-(1H-indol-3-yl)propanoate hydrochloride (**6**)*

White solid, 4.63 g (100%). ^1H NMR (400 MHz, CD_3OD): δ 7.53 (1H, d, *J*=7.9 Hz, Ar-H), 7.39 (1H, d, *J*=8.1 Hz, Ar-H), 7.22 (1H, s, Ar-H), 7.13 (1H, t, *J*=7.0 Hz, Ar-H), 7.06 (1H, t, *J*=7.0 Hz, Ar-H), 4.33 (1H, dd, *J*=7.2, 5.6 Hz, $C\underline{H}NH_3$), 3.77 (3H, s, OCH_3), 3.45 (1H, dd, *J*=15.0, 5.6 Hz, $C\underline{H}_AH_BCHN$), 3.37 (1H, dd, *J*=15.0, 7.2 Hz, $CH_A\underline{H}_BCHN$). ^{13}C NMR (100 MHz, CD_3OD): δ 171.3 (CO), 138.8 (Ar-C), 128.7 (Ar-C), 126.2 (Ar-C), 123.4 (Ar-C), 120.8 (Ar-C), 119.3 (Ar-C), 113.2 (Ar-C), 107.9 (Ar-C), 55.1 ($CHNH_3$), 54.1 (OCH_3), 28.0 ($\underline{C}H_2CHN$). The ^1H NMR spectrum is in agreement with the literature [54].

*7.16. (*S*)-Methyl 2-(2-(acetylthio)acetamido)-3-(1*H*-indol-3-yl)propanoate (**10a**)*

Orange oil, 1.44 g (96%). IR ν_{max}: 3349, 2954, 1737 (CO_2), 1680 (COS), 1662 (CON), 1524, 1357, 1222, 847 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.23 (1H, s, indole-NH), 7.52 (1H, d, *J*=8.1 Hz, Ar-H), 7.34 (1H, d, *J*=8.5 Hz, Ar-H), 7.18 (1H, t, *J*=7.9 Hz, Ar-H), 7.11 (1H, t, *J*=7.2 Hz, Ar-H), 7.00 (1H, d, *J*=2.4 Hz, Ar-H), 6.67 (1H, d, *J*=8.8 Hz, CHN<u>H</u>), 4.89 (1H, dt, *J*=7.0, 5.5 Hz, C<u>H</u>NH), 3.69 (3H, s, OCH_3), 3.54 (1H, d, *J*=15.0 Hz, C<u>H</u>$_A$CH$_B$S), 3.50 (1H, d, *J*=15.0 Hz, CH$_A$C<u>H</u>$_B$S)), 3.29 (2H, m, C<u>H</u>$_2$CHN), 2.24 (3H, s, CH_3CO). ^{13}C NMR (125 MHz, $CDCl_3$): δ 195.2 (COS), 172.1 (CO_2), 167.8 (CON), 136.2 (Ar-C), 127.7 (Ar-C), 123.0 (Ar-C), 122.3 (Ar-C), 119.8 (Ar-C), 118.7 (Ar-C), 111.4 (Ar-C), 109.9 (Ar-C), 53.3 (CHN), 52.6 (CH_3O), 33.0 (CH_2S), 30.2 (<u>C</u>H$_2$CHN), 27.5 (<u>C</u>H$_3$CO). HRMS calculated for $C_{16}H_{18}N_2NaO_4S$ $[M+Na]^+$ 357.0879, found 357.0886.

*7.17. (*S*)-3-(1*H*-Indol-3-yl)-2-(2-mercaptoacetamido)propanoic acid (**14a**)*

Yellow waxy solid, 0.47 g (70%). IR ν_{max}: 3379, 2929, 2550 (SH), 1717 (CO_2), 1618 (CON), 1530, 1214, 742 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD): δ 7.55 (1H, d, *J*=8.1 Hz, Ar-H), 7.31 (1H, d, *J*=8.1 Hz, Ar-H), 7.13-6.92 (3H, m, Ar-H), 4.72 (1H, dd, *J*=7.0, 5.1 Hz, C<u>H</u>NH), 3.36 (1H, dd, *J*=14.7, 5.2 Hz, C<u>H</u>$_A$H$_B$CHN), 3.22 (1H, dd, *J*=14.7, 7.2 Hz, CH$_A$<u>H</u>$_B$CHN), 3.12 (2H, s, CH_2S). ^{13}C NMR (75 MHz, CD_3OD): δ 175.3 (CO_2), 173.3 (CON), 138.5 (Ar-C), 129.4 (Ar-C), 125.0 (Ar-C), 122.9 (Ar-C), 120.3 (Ar-C), 119.8 (Ar-C), 112.8 (Ar-C), 111.2 (Ar-C), 55.4 (CHNH), 28.8 (CH_2SH), 28.6 (<u>C</u>H$_2$CHN). HRMS calculated for $C_{13}H_{13}N_2O_3S$ $[M-H]^-$ 277.0652, found 277.0658.

*7.18. (*S*)-Methyl 2-(3-(acetylthio)propanamido)-3-(1*H*-indol-3-yl)propanoate (**10b**)*

Brown viscous oil, 1.49 g (95%). IR ν_{max}: 3323, 2951, 1737 (CO_2), 1680 (COS), 1658 (CON), 1527, 1435, 1216, 745 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 8.12 (1H, s, indole-NH), 7.50 (1H, d, *J*=8.1 Hz, Ar-H), 7.34 (1H, d, *J*=9.6 Hz, Ar-H), 7.18 (1H, t, *J*=6.9 Hz, Ar-H), 7.09 (1H, t, *J*=7.8 Hz, Ar-H), 6.97 (1H, d, *J*=2.4 Hz, Ar-H), 6.00 (1H, d, *J*=7.8 Hz, NHCO), 4.94 (1H, dt, *J*=7.8, 5.4 Hz, C<u>H</u>NH), 3.68 (3H, s, OCH_3), 3.30 (2H, dd, *J*=5.1, 2.6 Hz, C<u>H</u>$_2$CHN), 3.09 (2H, t, *J*=6.9 Hz, CH_2S), 2.45 (2H, t, *J*=6.9 Hz, CH_2CO), 2.30 (3H, s, CH_3CO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 195.9 (COS), 172.2 (CON), 170.2 (CO_2), 136.1 (Ar-C), 127.7 (Ar-C), 122.7 (Ar-C), 122.3 (Ar-C), 119.8 (Ar-C), 118.5 (Ar-C), 111.3 (Ar-C), 110.0 (Ar-C), 53.0 (CHN), 52.4 (CH_3O), 36.1 (CH_2S), 30.5 (<u>C</u>H$_2$CO), 27.6 (OCH_3), 24.6 (<u>C</u>H$_2$CHN). HRMS calculated for $C_{17}H_{20}N_2NaO_4S$ $[M+Na]^+$ 371.1052, found 371.1036.

*7.19. (*S*)-3-(1*H*-Indol-3-yl)-2-(3-mercaptopropanamido)propanoic acid (**14b**)*

Brown waxy solid, 0.47 g (67%). IR ν_{max}: 3379, 2940, 2550 (SH), 1718 (CO_2), 1625 (CON), 1525, 1210, 742 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6)**:** δ 10.82 (1H, s, indole-NH), 8.20 (1H, d, *J*=7.7 Hz, NHCO), 7.52 (1H, d, *J*=8.1 Hz, Ar-H), 7.31 (1H, d, *J*=7.7 Hz, Ar-H), 7.13 (1H, d, *J*=2.2 Hz, Ar-H), 7.05 (1H, dt, *J*=7.3 Hz, 1.3 Hz, Ar-H), 6.96 (1H, dt, *J*=7.3, 1.3 Hz, Ar-H), 4.47 (1H, dt, *J*=7.8, 5.4 Hz, C<u>H</u>NH), 3.16 (1H, dd, *J*=15.0, 5.0 Hz, C<u>H</u>$_A$H$_B$CHN), 2.99 (1H, dd, *J*=15.0, 9.0 Hz, CH$_A$<u>H</u>$_B$CHN), 2.69-2.52 (2H, m, CH_2S), 2.44-2.30 (2H, m, CH_2CO), 2.17 (1H, t, *J*=9.3 Hz, SH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 173.4 (CO_2), 170.2 (CON), 136.0 (Ar-C), 127.2 (Ar-C), 123.5 (Ar-C), 120.9 (Ar-C), 118.3 (Ar-C), 118.1 (Ar-C), 111.3 (Ar-C), 109.9 (Ar-C), 52.9 (CHN), 30.7 (<u>C</u>H$_2$CO), 27.1 (<u>C</u>H$_2$CHN), 19.8 (CH_2SH). HRMS calculated for $C_{14}H_{15}N_2O_3S$ $[M-H]^-$ 291.0809, found 291.0812.

*7.20. (*S*)-Methyl 2-(4-(acetylthio)butanamido)-3-(1*H*-indol-3-yl)propanoate (**10c**)*

Brown viscous oil, 1.47 g (90%). IR ν_{max}: 3313, 2936, 1736 (CO_2), 1680 (COS), 1652 (CON), 1523, 1435, 1214, 744 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 8.20 (1H, bs, indole-NH), 7.50 (1H, d, *J*=7.8 Hz,

Ar-H), 7.34 (1H, d, *J*=7.4 Hz, Ar-H), 7.17 (1H, t, *J*=7.1 Hz, Ar-H), 7.10 (1H, t, *J*=7.1 Hz, Ar-H), 6.97 (1H, d, *J*=2.3 Hz, Ar-H), 6.05 (1H, d, *J*=7.7 Hz, NHCO), 4.93 (1H, dt, *J*=7.8, 5.5 Hz, C<u>H</u>NH), 3.68 (3H, s, OCH_3), 3.36-3.23 (2H, dd, *J*=8.4, 5.6 Hz, C<u>H_2</u>CHN), 2.82 (2H, dt, *J*=2.8, 7.3 Hz, CH_2S), 2.29 (3H, s, CH_3CO), 2.17 (2H, t, *J*=7.6 Hz, CH_2CO), 1.85 (2H, quintet, *J*=7.2 Hz, $CH_2C\underline{H_2}CH_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.9 (COS), 172.4 (CON), 171.5 (CO_2), 136.1 (Ar-C), 127.7 (Ar-C), 122.7 (Ar-C), 122.3 (Ar-C), 119.7 (Ar-C), 118.5 (Ar-C), 111.3 (Ar-C), 110.1 (Ar-C), 52.9 (CHN), 52.4 (CH_3O), 34.9 (<u>C</u>H_2CO), 30.6 (CH_2S), 28.3 (<u>C</u>H_3CO), 27.6 (<u>C</u>H_2CHN), 25.3 ($CH_2\underline{C}H_2CH_2$). HRMS calculated for $C_{18}H_{22}N_2NaO_4S$ $[M+Na]^+$ 385.1192, found 385.1203.

*7.21. (S)-3-(1H-Indol-3-yl)-2-(4-mercaptobutanamido)propanoic acid (**14c**)*

Brown viscous oil, 0.48 g (65%). IR ν_{max}: 3287, 2971, 2901, 2550 (SH), 1735 (CO_2), 1634 (CON), 1527, 1230, 1066, 743 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD**):** δ 7.54 (1H, t, *J*=7.2 Hz, Ar-H), 7.31 (1H, t, *J*=7.2 Hz, Ar-H), 7.11-7.03 (2H, m, Ar-H), 6.99 (1H, q, *J*=7.0 Hz, Ar-H), 4.75 (1H, dt, *J*=7.8, 5.5 Hz, C<u>H</u>NH), 3.36 (1H, dd, *J*=15.0, 4.5 Hz, C<u>H_A</u>H_BCHN), 3.13 (1H, dd, *J*=15.0, 8.3 Hz, CH_A<u>H_B</u>CHN), 2.42 (2H, t, *J*=7.2 Hz, CH_2S), 2.27-2.21 (2H, m, CH_2CO), 1.81 (2H, quintet, *J*=7.2 Hz, $CH_2C\underline{H_2}CH_2$). ^{13}C NMR (125 MHz, CD_3OD): δ 175.3 (CO_2), 175.2 (CON), 138.0 (Ar-C), 128.8 (Ar-C), 124.4 (Ar-C), 122.4 (Ar-C), 119.8 (Ar-C), 119.2 (Ar-C), 112.3 (Ar-C), 111.1 (Ar-C), 54.6 (CHN), 38.3 (<u>C</u>H_2CHN), 35.1 (<u>C</u>H_2CO), 28.5 (CH_2S), 26.1 ($CH_2\underline{C}H_2CH_2$). HRMS calculated for $C_{15}H_{17}N_2O_3S$ $[M-H]^-$ 305.0965, found 305.0960.

*7.22. (S)-Methyl 2-amino-3-phenylpropanoate hydrochloride (**7**)*

White solid, 3.92 g (100%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.83 (3H, bs, NH_3), 7.34-7.22 (5H, m, Ar-H), 4.20 (1H, dd, *J*=7.6, 5.5 Hz, C<u>H</u>NH_3), 3.63 (3H, s, OCH_3), 3.23 (1H, dd, *J*=13.4, 5.0 Hz, C<u>H_A</u>H_BCHN), 3.09 (1H, dd, *J*=13.4, 7.4 Hz, CH_A<u>H_B</u>CHN). ^{13}C NMR (75 MHz, DMSO-d_6): δ 169.3

(CO), 134.8 (Ar-C), 129.4 (Ar-C), 128.6 (Ar-C), 127.2 (Ar-C), 53.3 (CHN), 52.5 (OCH_3), 35.8 ($\underline{C}H_2CHN$). The NMR spectra are in agreement with the literature [55].

*7.23. (S)-Methyl 2-(2-(acetylthio)acetamido)-3-phenylpropanoate (**11a**)*

Orange oil, 1.30 g (98%). IR ν_{max}: 3317, 2953, 1740 (CO_2), 1680 (COS), 1662 (CON), 1526, 1215, 846 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.31-7.21 (3H, m, Ar-H), 7.10-7.06 (2H, m, Ar-H), 6.63 (1H, d, *J*=7.3 Hz, NH), 4.81 (1H, dd, *J*=7.6, 6.2 Hz, C$\underline{H}$NH), 3.72 (3H, s, OCH_3), 3.57 (1H, d, *J*=14.9 Hz, C$\underline{H}_A$H$_B$S), 3.49 (1H, d, *J*=14.9 Hz, CH$_A\underline{H}_B$S), 3.15 (1H, dd, *J*=5.6, 14.0 Hz, C$\underline{H}_A$H$_B$CHN), 3.06 (1H, dd, *J*=6.2, 14.0 Hz, CH$_A\underline{H}_B$CHN), 2.34 (3H, s, CH_3CO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 195.2 (COS), 171.5 (CO_2), 167.6 (CON), 135.6 (Ar-C), 129.3 (Ar-C), 128.5 (Ar-C), 127.1 (Ar-C), 53.4 (CHN), 52.4 (OCH_3), 37.6 ($\underline{C}H_2CHN$), 32.7 (CH_2S), 30.1 ($\underline{C}H_3CO$). HRMS calculated for $C_{14}H_{17}NNaO_4S$ $[M+Na]^+$ 318.0770, found 318.0769.

*7.24. (S)-2-(2-Mercaptoacetamido)-3-phenylpropanoic acid (**15a**)*

Brown oil, 0.45 g (79%). IR ν_{max}: 3318, 2947, 2565 (SH), 1719 (CO_2), 1632 (CON), 1526, 1208, 699 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 8.36 (1H, d, *J*=9.0 Hz, NH), 7.27-7.17 (5H, m, Ar-H), 4.46-4.41 (1H, m, C$\underline{H}$NH), 3.42 (2H, s, CH_2S), 3.05 (1H, dd, *J*=14.1, 5.3 Hz, C$\underline{H}_A$H$_B$CHN), 2.87 (1H, dd, *J*=14.1, 8.4 Hz, CH$_A\underline{H}_B$CHN). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.7 (CO_2), 167.8 (CON), 137.3 (Ar-C), 129.2 (Ar-C), 128.2 (Ar-C), 126.5 (Ar-C), 53.8 (CHN), 41.8 ($\underline{C}H_2CHN$), 36.8 (CH_2S). HRMS calculated for $C_{11}H_{12}NO_3S$ $[M-H]^-$ 238.0543, found 238.0543.

*7.25. (S)-Methyl 2-(3-(acetylthio)propanamido)-3-phenylpropanoate (**11b**)*

Tan solid, 1.34 g (96%). IR ν_{max}: 3292, 2953, 1728 (CO_2), 1696 (COS), 1642 (CON), 1545, 1131, 696 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.32-7.23 (3H, m, Ar-H), 7.12-7.08 (2H, m, Ar-H), 6.02 (1H, d,

J=7.0 Hz, NH), 4.89 (1H, dt, *J*=7.8, 5.8 Hz, C<u>H</u>NH), 3.73 (3H, s, OCH_3), 3.20-3.05 (4H, m, C<u>H_2</u>CHN and CH_2S), 2.51-2.46 (2H, m, $COCH_2$), 2.32 (3H, s, CH_3CO). ^{13}C NMR (75 MHz, $CDCl_3$): δ 195.9 (COS), 171.9 (CONH), 170.2 (CO_2), 135.7 (Ar-C), 129.2 (Ar-C), 128.6 (Ar-C), 127.1 (Ar-C), 53.1 (CHN), 52.3 (OCH_3), 37.8 (<u>C</u>H_2CHN), 36.0 (CH_2S), 30.5 (CO<u>C</u>H_2), 24.7 (<u>C</u>H_3CO). HRMS calculated for $C_{15}H_{19}NNaO_4S$ $[M+Na]^+$ 332.0937, found 332.0919. The NMR spectra are in agreement with those reported for the enantiomer [40].

*7.26. (S)-2-(3-Mercaptopropanamido)-3-phenylpropanoic acid (**15b**)*

Brown oil, 0.43 g (70%). IR ν_{max}: 3318, 2973, 2565 (SH), 1712 (CO_2), 1656 (CON), 1089, 1047, 879 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD): δ 7.27-7.18 (5H, m, Ar-H), 4.68 (1H, dd, *J*=9.1, 5.1, C<u>H</u>NH), 3.20 (1H, dd, *J*=14.1, 4.8 Hz, C<u>H_A</u>H_BCHN), 2.93 (1H, dd, *J*=14.1, 9.3 Hz, CH_A<u>H_B</u>CHN), 2.81-2.76 (2H, m, CH_2S), 2.55-2.51 (2H, m, CH_2CO). ^{13}C NMR (100 MHz, CD_3OD): δ 175.2 (CO_2), 174.1 (CON), 138.9 (Ar-C), 130.8 (Ar-C), 130.0 (Ar-C), 128.3 (Ar-C), 55.5 (CHN), 38.9 (<u>C</u>H_2CHN), 36.7 (<u>C</u>H_2CO), 35.4 (CH_2S). HRMS calculated for $C_{24}H_{28}N_2NaO_6S_2$ $[2M+Na]^+$ 527.1281, found 527.1275. The ^{1}H NMR spectrum is in agreement with that reported for the enantiomer [40].

*7.27. (S)-Methyl 2-(4-(acetylthio)butanamido)-3-phenylpropanoate (**11c**)*

Brown oil, 1.31 g (90%). IR ν_{max}: 3333, 2954, 1737 (CO_2), 1680 (COS), 1658 (CON), 1531, 1221, 847 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.28-7.22 (3H, m, Ar-H), 7.07 (2H, d, *J*=7.0 Hz, Ar-H), 6.01 (1H, d, *J*=7.7 Hz, NH), 4.87 (1H, dd, *J*=9.7, 5.0 Hz, C<u>H</u>NH), 3.71 (3H, s, OCH_3), 3.15 (1H, dd, *J*=14.0, 5.5 Hz, C<u>H_A</u>H_BCHN), 3.05 (1H, dd, *J*=14.0, 6.5 Hz, CH_A<u>H_B</u>CHN), 2.87-2.80 (2H, m, CH_2S), 2.30 (3H, s, CH_3CO), 2.21 (2H, dt, *J*=7.3, 2.5 Hz, CH_2CO), 1.85 (2H, quintet, *J*=7.2 Hz, CH_2C<u>H_2</u>CH_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 195.9 (COS), 172.1 (CON), 171.4 (CO_2), 135.9 (Ar-C), 129.2 (Ar-C), 128.6 (Ar-C), 127.1 (Ar-C), 53.0 (CHN), 52.3 (OCH_3), 37.9 (<u>C</u>H_2CHN), 34.8 (<u>C</u>H_2CO), 30.6 (CH_2S), 28.3

($\underline{C}H_3CO$), 25.4 ($CH_2\underline{C}H_2CH_2$). HRMS calculated for $C_{16}H_{21}NNaO_4S$ $[M+Na]^+$ 346.1083, found 346.1080.

*7.28. (S)-2-(4-mercaptobutanamido)-3-phenylpropanoic acid (**15c**)*

Brown viscous oil, 0.42 g (65%). IR ν_{max}: 3318, 2941, 2565 (SH), 1718 (CO_2), 1633 (CON), 1531, 1219, 699 cm^{-1}. ^1H NMR (500 MHz, CD_3OD): δ 7.27-7.17 (5H, m, Ar-H), 4.68 (1H, dd, *J*=9.7, 5.0 Hz, C$\underline{H}$NH), 3.22 (1H, dd, *J*=14.0, 4.7 Hz, C$\underline{H}_A$H$_B$CHN), 2.91 (1H, dd, *J*=14.0, 10.0 Hz, CH$_A\underline{H}_B$CHN), 2.48 (2H, t, *J*=7.3 Hz, CH_2CO), 2.24 (2H, dt, *J*=4.7, 7.3 Hz, CH_2S), 1.84 (2H, quintet, *J*=7.0 Hz, $CH_2\underline{C}H_2CH_2$). ^{13}C NMR (125 MHz, CD_3OD): δ 175.1 (CO_2), 174.8 (CON), 138.5 (Ar-C), 130.2 (Ar-C), 129.5 (Ar-C), 127.8 (Ar-C), 54.9 (CHNH), 38.38 ($\underline{C}H_2CHN$), 38.36 (CH_2SH), 35.1 ($\underline{C}H_2CO$), 26.2 ($CH_2\underline{C}H_2CH_2$). HRMS calculated for $C_{13}H_{17}NNaO_3S$ $[M+Na]^+$ 290.0821, found 290.0827.

*7.29. (S)-Methyl 2-amino-3-(4-hydroxyphenyl)propanoate hydrochloride (**8**)*

White solid, 4.21 g (100%). ^1H NMR (300 MHz, DMSO-d_6): δ 9.47 (1H, bs, OH), 8.63 (3H, bs, NH_3), 7.00 (2H, d, *J*=8.5 Hz, Ar-H), 6.71 (2H, d, *J*=8.5 Hz, Ar-H), 4.13 (1H, t, *J*=5.8 Hz, C$\underline{H}NH_3$), 3.65 (3H, s, OCH_3), 3.07 (1H, dd, *J*=15.0, 5.6 Hz, C$\underline{H}_A$H$_B$CHN), 2.97 (1H, dd, *J*=15.0, 7.2 Hz, CH$_A\underline{H}_B$CHN). ^{13}C NMR (75 MHz, DMSO-d_6): δ 169.4 (CO_2), 156.7 (Ar-C), 130.3 (Ar-C), 124.3 (Ar-C), 115.4 (Ar-C), 53.4 (CHN), 52.5 (CH_3O), 35.0 ($\underline{C}H_2CHN$). The NMR spectra are in agreement with the literature [56].

*7.30. (S)-Methyl 2-(2-(acetylthio)acetamido)-3-(4-hydroxyphenyl)propanoate (**12a**)*

Pale yellow oil, 1.35 g (95%). IR ν_{max}: 3675, 3304, 2989, 2901, 1740 (CO_2), 1698 (COS), 1655 (CON), 1510, 1233, 1109, 737 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 6.91 (2H, d, *J*=8.8 Hz, Ar-H), 6.70 (2H, d, *J*=8.4 Hz, Ar-H), 6.66 (1H, d, *J*=8.9 Hz, NH), 4.82-4.70 (1H, dt, *J*=7.8, 5.5 Hz, C$\underline{H}$NH), 3.71 (3H, s, OCH_3), 3.55 (1H, d, *J*=15.0 Hz, C$\underline{H}_A$H$_B$S), 3.49 (1H, d, *J*=15.0 Hz, CH$_A\underline{H}_B$S), 3.06 (1H, dd, *J*=14.1, 5.7

Hz, C$\underline{H}_A$H$_B$CHN), 2.93 (1H, dd, *J*=14.1, 6.7 Hz, CH$_A\underline{H}_B$CHN), 2.33 (3H, s, CH$_3$CO). ^{13}C NMR (75 MHz, CDCl$_3$): δ 195.4 (COS), 171.7 (CO$_2$), 168.0 (CON), 155.2 (Ar-C), 130.3 (Ar-C), 127.0 (Ar-C), 115.5 (Ar-C), 53.6 (CHN), 52.5 (OCH$_3$), 36.9 (C$\underline{H}_2$CHN), 32.7 (CH$_2$S), 30.2 (C$\underline{H}_3$CO). HRMS calculated for C$_{14}$H$_{17}$NNaO$_5$S [M+Na]$^+$ 334.0720, found 334.0728.

*7.31. (S)-3-(4-Hydroxyphenyl)-2-(2-mercaptoacetamido)propanoic acid (**16a**)*

Pale yellow viscous oil, 0.40 g (65%). IR ν_{max}: 3314, 2972, 2550 (SH), 1721 (CO$_2$), 1653 (CON), 1513, 1186 cm^{-1}. ^{1}H NMR (400 MHz, CD$_3$OD): δ 7.03 (2H, d, *J*=8.4 Hz, Ar-H), 6.69 (2H, d, *J*=8.4 Hz, Ar-H), 4.59 (1H, dd, *J*=8.1, 5.2 Hz, C$\underline{H}$NH), 3.13 (2H, s, CH$_2$S), 3.10 (1H, dd, *J*=14.1, 5.2 Hz, C$\underline{H}_A$H$_B$CHN), 2.91 (1H, dd, *J*=14.1, 8.3 Hz, CH$_A\underline{H}_B$CHN). ^{13}C NMR (100 MHz, CD$_3$OD): δ 174.5 (CO$_2$), 172.9 (CON), 157.4 (Ar-C), 131.4 (Ar-C), 128.7 (Ar-C), 116.2 (Ar-C), 55.4 (CHN), 37.5 (C$\underline{H}_2$CHN), 28.0 (CH$_2$S). HRMS calculated for C$_{11}$H$_{12}$NO$_4$S [M-H]$^-$ 254.0493, found 254.0492.

*7.32. (S)-Methyl 2-(3-(acetylthio)propanamido)-3-(4-hydroxyphenyl)propanoate (**12b**)*

Brown viscous oil, 1.35 g (92%). IR ν_{max}: 3675, 3317, 2974, 1736 (CO$_2$), 1680 (COS), 1652 (CON), 1514, 1221, 844 cm^{-1}. ^{1}H NMR (500 MHz, CDCl$_3$): δ 6.91 (2H, d, *J*=8.5 Hz, Ar-H), 6.70 (2H, d, *J*=8.6 Hz, Ar-H), 6.22 (1H, d, *J*=8.0 Hz, NH), 4.83 (1H, dt, *J*=7.7, 6.1 Hz, C$\underline{H}$NH), 3.71 (3H, s, OCH$_3$), 3.07-3.02 (3H, m, CH$_2$S and C$\underline{H}_A$H$_B$CHN), 2.95 (1H, dd, *J*=14.2, 6.1 Hz, CH$_A\underline{H}_B$CHN), 2.51-2.42 (2H, m, CH$_2$CO), 2.29 (3H, s, CH$_3$CO). ^{13}C NMR (125 MHz, CDCl$_3$): δ 196.4 (COS), 172.1 (CON), 170.8 (CO$_2$), 155.4 (Ar-C), 130.3 (Ar-C), 126.9 (Ar-C), 115.5 (Ar-C), 53.3 (CHN), 52.4 (OCH$_3$), 37.0 (C$\underline{H}_2$CHN), 36.0 (CH$_2$S), 30.5 (C$\underline{H}_2$CO), 24.7 (CH$_3$CO). HRMS calculated for C$_{15}$H$_{19}$NNaO$_5$S [M+Na]$^+$ 348.0876, found 348.0873.

*7.33. (S)-3-(4-Hydroxyphenyl)-2-(3-mercaptopropanamido)propanoic acid (**16b**)*

Pale yellow viscous oil, 0.41 g (63%). IR ν_{max}: 3314, 2973, 2550 (SH), 1722 (CO_2), 1654 (CON), 1514, 1190 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 9.17 (1H, s, OH), 8.16 (1H, d, *J*=9.5 Hz, NH), 7.00 (2H, d, *J*=8.0 Hz, Ar-H), 6.63 (2H, *J*=8.3 Hz, Ar-H), 4.40-4.26 (1H, m, C<u>H</u>NH), 2.91 (1H, dd, *J*=13.8, 5.2 Hz, C<u>H</u>$_A$H$_B$CHN), 2.72 (1H, dd, *J*=13.8, 9.7 Hz, CH$_A$<u>H</u>$_B$CHN), 2.56 (2H, t, *J*=7.5 Hz, CH_2S), 2.37 (2H, t, *J*=7.5 Hz, CH_2CO), 2.14 (1H, t, *J*=8.0 Hz, SH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.0 (CO_2), 170.2 (CON), 155.9 (Ar-C), 130.0 (Ar-C), 127.6 (Ar-C), 114.9 (Ar-C), 53.7 (CHN), 38.1 (<u>C</u>H_2CHN), 36.0 (<u>C</u>H_2CO), 21.0 (CH_2S). (HRMS calculated for $C_{12}H_{14}NO_4S$ $[M-H]^-$ 268.0649, found 268.0644.

*7.34. (S)-Methyl 2-(4-(acetylthio)butanamido)-3-(4-hydroxyphenyl)propanoate (**12c**)*

Brown viscous oil, 1.37 g (90%). IR ν_{max}: 3676, 3332, 2972, 1737 (CO_2), 1680 (COS), 1653 (CON), 1514, 1223, 841 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 6.93 (2H, d, *J*=8.4 Hz, Ar-H), 6.71 (2H, d, *J*=8.4 Hz, Ar-H), 6.12 (1H, d, *J*=8.1 Hz, NH), 4.84 (1H, dt, *J*=7.5, 5.5 Hz, C<u>H</u>NH), 3.71 (3H, s, OCH_3), 3.07 (1H, dd, *J*=13.0, 5.7 Hz, C<u>H</u>$_A$H$_B$CHN), 2.95 (1H, dd, *J*=14.0, 6.7 Hz, CH$_A$<u>H</u>$_B$CHN), 2.86-2.76 (2H, m, CH_2S), 2.31 (3H, s, CH_3CO), 2.24-2.19 (2H, m, CH_2CO), 1.84 (2H, quintet, *J*=7.2 Hz, $CH_2C\underline{H}_2CH_2$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 196.3 (COS), 172.3 (CON), 171.9 (CO_2), 155.3 (Ar-C), 130.3 (Ar-C), 127.3 (Ar-C), 115.5 (Ar-C), 53.2 (CHN), 52.4 (OCH_3), 37.1 (<u>C</u>H_2CHN), 34.8 (<u>C</u>H_2CO), 30.6 (CH_2S), 28.2 (<u>C</u>H_3CO), 25.4 ($CH_2\underline{C}H_2CH_2$). HRMS calculated for $C_{16}H_{21}NNaO_5S$ $[M+Na]^+$ 362.1033, found 362.1042.

*7.35. (S)-3-(4-Hydroxyphenyl)-2-(4-mercaptobutanamido)propanoic acid (**16c**)*

Brown viscous oil, 0.41 g, 60%. IR ν_{max}: 3314, 2973, 2550 (SH), 1700 (CO_2), 1614 (CON), 1379, 1045 cm^{-1}. ^1H NMR (500 MHz, CD_3OD): δ 7.03 (2H, d, *J*=8.5 Hz, Ar-H), 6.69 (2H, d, *J*=8.5 Hz, Ar-H), 4.64-4.58 (1H, m, C<u>H</u>NH), 3.11 (1H, dd, *J*=14.0, 4.9 Hz, C<u>H</u>$_A$H$_B$CHN), 2.82 (1H, dd, *J*=14.0, 9.6 Hz, CH$_A$<u>H</u>$_B$CHN), 2.51 (2H, t, *J*=6.9 Hz, CH_2CO), 2.30-2.22 (2H, m, CH_2S), 1.87 (2H, quintet, *J*=7.2 Hz, $CH_2C\underline{H}_2CH_2$). ^{13}C NMR (125 MHz, CD_3OD): δ 175.2 (CO_2), 175.0 (CON), 157.3 (Ar-C), 131.2 (Ar-C), 129.1 (Ar-C), 116.2 (Ar-C), 55.2 (CHN), 37.6 ($\underline{C}H_2CHN$), 35.2 ($\underline{C}H_2CO$), 31.2 (CH_2S), 26.2 ($CH_2\underline{C}H_2CH_2$). HRMS calculated for $C_{13}H_{16}NO_4S$ $[M-H]^-$ 282.0806, found 282.0795.

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Appendix A. Supplementary data

Details of kinetic assays; MolDock scores for binding of inhibitors to the IMP-1 enzyme; NMR spectra of synthesized compounds.

8. References

[1] D.T. King, N.C.J. Strynadka, Targeting metallo-β-lactamase enzymes in antibiotic resistance, Future Med. Chem., 5 (2013) 1243-1263.
[2] S.M. Drawz, R.A. Bonomo, Three decades of β-lactamase inhibitors, Clin. Microbiol. Rev., 23 (2010) 160-201.
[3] P. Vella, M. Miraula, E. Phelan, E.W.W. Leung, F. Ely, D.L. Ollis, R.P. McGeary, G. Schenk, N. Mitic, Identification and characterization of an unusual metallo-β-lactamase from *Serratia proteamaculans*, JBIC, J. Biol. Inorg. Chem., 18 (2013) 855-863.
[4] C.-F.D. Hou, E.K. Phelan, M. Miraula, D.L. Ollis, G. Schenk, N. Mitic, Unusual metallo-β-lactamases may constitute a new subgroup in this family of enzymes, Am. J. Mol. Biol., 4 (2014) 11-15.
[5] C. Bebrone, Metallo-β-lactamases (classification, activity, genetic organization, structure, zinc coordination) and their superfamily, Biochem. Pharmacol., 74 (2007) 1686-1701.
[6] M.W. Crowder, J. Spencer, A.J. Vila, Metallo-β-lactamases: Novel weaponry for antibiotic resistance in bacteria, Acc. Chem. Res., 39 (2006) 721-728.
[7] J. Moran-Barrio, J.M. Gonzalez, M.N. Lisa, A.L. Costello, P.M. Dal, P. Carloni, B. Bennett, D.L. Tierney, A.S. Limansky, A.M. Viale, A.J. Vila, The Metallo-β-lactamase GOB Is a Mono-Zn(II) Enzyme with a Novel Active Site, J. Biol. Chem., 282 (2007) 18286-18293.
[8] R. Paul-Soto, R. Bauer, J.M. Frere, M. Galleni, W. Meyer-Klaucke, H. Nolting, G.M. Rossolini, S.D. de, M. Hernandez-Valladares, M. Zeppezauer, H.W. Adolph, Mono- and binuclear Zn^{2+}-β-lactamase. Role of the conserved cysteine in the catalytic mechanism, J. Biol. Chem., 274 (1999) 13242-13249.
[9] E.K. Phelan, M. Miraula, C. Selleck, D.L. Ollis, G. Schenk, N. Mitic, Metallo-β-lactamases: a major threat to human health, Am. J. Mol. Biol., 4 (2014) 89-104.
[10] N. Mitić, M. Miraula, C. Selleck, K.S. Hadler, E. Uribe, M.M. Pedroso, G. Schenk, Chapter Three - Catalytic Mechanisms of Metallohydrolases Containing Two Metal Ions, in: Z.C. Christo (Ed.) Advances in Protein Chemistry and Structural Biology, Academic Press, 2014, pp. 49-81.
[11] A. Felici, G. Amicosante, A. Oratore, R. Strom, P. Ledent, B. Joris, L. Fanuel, J.M. Frere, An overview of the kinetic parameters of class B β-lactamases, Biochem. J., 291 (1993) 151-155.
[12] P. Lassaux, M. Hamel, M. Gulea, H. Delbruck, P.S. Mercuri, L. Horsfall, D. Dehareng, M. Kupper, J.-M. Frere, K. Hoffmann, M. Galleni, C. Bebrone, Mercaptophosphonate Compounds as Broad-Spectrum Inhibitors of the Metallo-β-lactamases, J. Med. Chem., 53 (2010) 4862-4876.
[13] S. Ohsuka, Y. Arakawa, T. Horii, H. Ito, M. Ohta, Effect of pH on activities of novel β-lactamases and β-lactamase inhibitors against these β-lactamases, Antimicrob. Agents Chemother., 39 (1995) 1856-1858.
[14] J. Li, R.L. Nation, J.D. Turnidge, R.W. Milne, K. Coulthard, C.R. Rayner, D.L. Paterson, Colistin: the re-emerging antibiotic for multidrug-resistant gram-negative bacterial infections, Lancet Infect. Dis., 6 (2006) 589-601.
[15] D.M. Livermore, Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: Our worst nightmare?, Clin. Infect. Dis., 34 (2002) 634-640.
[16] R.P. McGeary, G. Schenk, L.W. Guddat, The applications of binuclear metallohydrolases in medicine: Recent advances in the design and development of novel drug leads for purple acid phosphatases, metallo-β-lactamases and arginases, Eur. J. Med. Chem., 76 (2014) 132-144.
[17] M.W. Walter, A. Felici, M. Galleni, R.P. Soto, R.M. Adlington, J.E. Baldwin, J.-M. Frère, M. Gololobov, C.J. Schofield, Trifluoromethyl alcohol and ketone inhibitors of metallo-β-lactamases, Bioorg. Med. Chem. Lett., 6 (1996) 2455-2458.

[18] Y. Hiraiwa, A. Morinaka, T. Fukushima, T. Kudo, Metallo-β-lactamase inhibitory activity of phthalic acid derivatives, Bioorg. Med. Chem. Lett., 19 (2009) 5162-5165.
[19] J.H. Toney, G.G. Hammond, P.M.D. Fitzgerald, N. Sharma, J.M. Balkovec, G.P. Rouen, S.H. Olson, M.L. Hammond, M.L. Greenlee, Y.-D. Gao, Succinic Acids as Potent Inhibitors of Plasmid-borne IMP-1 Metallo-β-lactamase, J. Biol. Chem., 276 (2001) 31913-31918.
[20] L. Nauton, R. Kahn, G. Garau, J.F. Hernandez, O. Dideberg, Structural Insights into the Design of Inhibitors for the L1 Metallo-β-lactamase from *Stenotrophomonas maltophilia*, J. Mol. Biol., 375 (2008) 257-269.
[21] L. Olsen, S. Jost, H.-W. Adolph, I. Pettersson, L. Hemmingsen, F.S. Jorgensen, New leads of metallo-β-lactamase inhibitors from structure-based pharmacophore design, Bioorg. Med. Chem., 14 (2006) 2627-2635.
[22] L. Feng, K.-W. Yang, L.-S. Zhou, J.-M. Xiao, X. Yang, L. Zhai, Y.-L. Zhang, M.W. Crowder, N-heterocyclic dicarboxylic acids: broad-spectrum inhibitors of metallo-β-lactamases with co-antibacterial effect against antibiotic-resistant bacteria, Bioorg. Med. Chem. Lett., 22 (2012) 5185-5189.
[23] Y. Hiraiwa, J. Saito, T. Watanabe, M. Yamada, A. Morinaka, T. Fukushima, T. Kudo, X-ray crystallographic analysis of IMP-1 metallo-β-lactamase complexed with a 3-aminophthalic acid derivative, structure-based drug design, and synthesis of 3,6-disubstituted phthalic acid derivative inhibitors, Bioorg. Med. Chem. Lett., 24 (2014) 4891-4894.
[24] M. Goto, T. Takahashi, F. Yamashita, A. Koreeda, H. Mori, M. Ohta, Y. Arakawa, Inhibition of the metallo-β-lactamase produced from *Serratia marcescens* by thiol compounds, Biol. Pharm. Bull., 20 (1997) 1136-1140.
[25] C. Mollard, C. Moali, C. Papamicael, C. Damblon, S. Vessilier, G. Amicosante, C.J. Schofield, M. Galleni, J.-M. Frère, G.C.K. Roberts, Thiomandelic Acid, a Broad Spectrum Inhibitor of Zinc β-Lactamases, J. Biol. Chem., 276 (2001) 45015-45023.
[26] G.G. Hammond, J.L. Huber, M.L. Greenlee, J.B. Laub, K. Young, L.L. Silver, J.M. Balkovec, K.D. Pryor, J.K. Wu, B. Leiting, D.L. Pompliano, J.H. Toney, Inhibition of IMP-1 metallo-β-lactamase and sensitization of IMP-1-producing bacteria by thioester derivatives, FEMS Microbiol. Lett., 179 (1999) 289-296.
[27] U. Heinz, R. Bauer, S. Wommer, W. Meyer-Klaucke, C. Papamichaels, J. Bateson, H.-W. Adolph, Coordination Geometries of Metal Ions in D- or L-Captopril-inhibited Metallo-β-lactamases, J. Biol. Chem., 278 (2003) 20659-20666.
[28] W.M. Hussein, S.S. Fatahala, Z.M. Mohamed, R.P. McGeary, G. Schenk, D.L. Ollis, M.S. Mohamed, Synthesis and kinetic testing of tetrahydropyrimidine-2-thione and pyrrole derivatives as inhibitors of the metallo-β-lactamase from *Klebsiella pneumonia* and *Pseudomonas aeruginosa*, Chem. Biol. Drug Des., 80 (2012) 500-515.
[29] Faridoon, W.M. Hussein, P. Vella, N. Ul Islam, D.L. Ollis, G. Schenk, R.P. McGeary, 3-Mercapto-1,2,4-triazoles and N-acylated thiosemicarbazides as metallo-β-lactamase inhibitors, Bioorg. Med. Chem. Lett., 22 (2012) 380-386.
[30] M.S. Mohamed, W.M. Hussein, R.P. McGeary, P. Vella, G. Schenk, R.H. Abd El-hameed, Synthesis and kinetic testing of new inhibitors for a metallo-β-lactamase from *Klebsiella pneumonia* and *Pseudomonas aeruginosa*, Eur. J. Med. Chem., 46 (2011) 6075-6082.
[31] A.M. Simm, E.J. Loveridge, J. Crosby, M.B. Avison, T.R. Walsh, P.M. Bennett, Bulgecin A: a novel inhibitor of binuclear metallo-β-lactamases, Biochem. J., 387 (2005) 585-590.
[32] M.W. Walter, M.H. Valladares, R.M. Adlington, G. Amicosante, J.E. Baldwin, J.-M. Frère, M. Galleni, G.M. Rossolini, C.J. Schofield, Hydroxamate Inhibitors of *Aeromonas hydrophila* AE036 Metallo-β-lactamase, Bioorg. Chem., 27 (1999) 35-40.

[33] J.H. Toney, P.M.D. 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, Antibiotic sensitization using biphenyl tetrazoles as potent inhibitors of *Bacteroides fragilis* metallo-β-lactamase, Chem. Biol., 5 (1998) 185-196.
[34] S. Siemann, D.P. Evanoff, L. Marrone, A.J. Clarke, T. Viswanatha, G.I. Dmitrienko, N-Arylsulfonyl Hydrazones as Inhibitors of IMP-1 Metallo-β-Lactamase, Antimicrob. Agents Chemother., 46 (2002) 2450-2457.
[35] S. Fukigai, J. Alba, S. Kimura, T. Iida, N. Nishikura, Y. Ishii, K. Yamaguchi, Nosocomial outbreak of genetically related IMP-1 β-lactamase-producing *Klebsiella pneumoniae* in a general hospital in Japan, Int. J. Antimicrob. Agents, 29 (2007) 306-310.
[36] K.G. Kerr, A.M. Snelling, *Pseudomonas aeruginosa*: a formidable and ever-present adversary, J. Hosp. Infect., 73 (2009) 338-344.
[37] A.P. Penteado, M. Castanheira, A.C.C. Pignatari, T. Guimaraes, E.M. Mamizuka, A.C. Gales, Dissemination of bla IMP-1-carrying integron In86 among *Klebsiella pneumoniae* isolates harboring a new trimethoprim resistance gene dfr23, Diagn. Microbiol. Infect. Dis., 63 (2009) 87-91.
[38] P. Vella, W.M. Hussein, E.W.W. Leung, D. Clayton, D.L. Ollis, N. Mitic, G. Schenk, R.P. McGeary, The identification of new metallo-β-lactamase inhibitor leads from fragment-based screening, Bioorg. Med. Chem. Lett., 21 (2011) 3282-3285.
[39] J.H. Toney, G.G. Hammond, P.M.D. Fitzgerald, N. Sharma, J.M. Balkovec, G.P. Rouen, S.H. Olson, M.L. Hammond, M.L. Greenlee, Y.-D. Gao, Succinic acids as potent inhibitors of plasmid-borne IMP-1 metallo-β-lactamase, J. Biol. Chem., 276 (2001) 31913-31918.
[40] B.M.R. Lienard, C. Papamicael, C.J. Schofield, G. Garau, O. Dideberg, L. Horsfall, P. Lassaux, M. Galleni, J.-M. Frere, A.I. Karsisiotis, C. Damblon, G.C.K. Roberts, Structural basis for the broad-spectrum inhibition of metallo-β-lactamases by thiols, Org. Biomol. Chem., 6 (2008) 2282 - 2294.
[41] E. Benary, Thiotetronic Acid and Derivatives, Ber. Dtsch. Chem. Ges., 46 (1913) 2103-2107.
[42] A.-H. Li, S. Moro, N. Forsyth, N. Melman, X.-d. Ji, K.A. Jacobson, Synthesis, CoMFA Analysis, and Receptor Docking of 3,5-Diacyl-2,4-Dialkylpyridine Derivatives as Selective A3 Adenosine Receptor Antagonists, J. Med. Chem., 42 (1999) 706-721.
[43] J.H. Hogg, I.R. Ollmann, A. Wetterholm, M. Blomster Andberg, J. Haeggstrom, B. Samuelsson, C.-H. Wong, Probing the activities and mechanisms of leukotriene A4 hydrolase with synthetic inhibitors, Chem. - Eur. J., 4 (1998) 1698-1713.
[44] N.A. Bindman, S.C. Bobeica, W.R. Liu, W.A. van der Donk, Facile Removal of Leader Peptides from Lanthipeptides by Incorporation of a Hydroxy Acid, J. Am. Chem. Soc., 137 (2015) 6975-6978.
[45] T. Hamada, M. Yatagai, Method for production of O-substituted tyrosine compounds, in, Ajinomoto, Co., Inc., Japan . 2005, pp. 8 pp.
[46] R. Thomsen, M.H. Christensen, MolDock: A New Technique for High-Accuracy Molecular Docking, J. Med. Chem., 49 (2006) 3315-3321.
[47] C. Bebrone, C. Moali, F. Mahy, S. Rival, J.D. Docquier, G.M. Rossolini, J. Fastrez, R.F. Pratt, J.-M. Frère, M. Galleni, CENTA as a Chromogenic Substrate for Studying β-Lactamases, Antimicrob. Agents Chemother., 45 (2001) 1868-1871.
[48] P.M. Hawkey, J. Xiong, H. Ye, H. Li, F.H. M'Zali, Occurrence of a new metallo-β-lactamase IMP-4 carried on a conjugative plasmid in *Citrobacter youngae* from the People's Republic of China, FEMS Microbiol. Lett., 194 (2001) 53-57.
[49] N. Laraki, N. Franceschini, G.M. Rossolini, P. Santucci, C. Meunier, P.E. De, G. Amicosante, J.M. Frere, M. Galleni, Biochemical characterization of the *Pseudomonas aeruginosa* 101/1477 metallo-β-lactamase IMP-1 produced by *Escherichia coli*, Antimicrob. Agents Chemother., 43 (1999) 902-906.

[50] D.K. Maiti, A. Banerjee, A Synthetic Amino Acid Residue Containing A New Oligopeptide-Based Photosensitive Fluorescent Organogel, Chem. - Asian. J., 8 (2013) 113-120.
[51] N. Boyer, P. Gloanec, G. De Nanteuil, P. Jubault, J.-C. Quirion, Synthesis of α,α-difluoro-β-amino esters or gem-difluoro-β-lactams as potential metallocarboxypeptidase inhibitors, Eur. J. Org. Chem., (2008) 4277-4295.
[52] Y. Wang, X.-L. Guan, P.-F. Wu, C.-M. Wang, H. Cao, L. Li, X.-J. Guo, F. Wang, N. Xie, F.-C. Jiang, J.-G. Chen, Multifunctional Mercapto-tacrine Derivatives for Treatment of Age-Related Neurodegenerative Diseases, J. Med. Chem., 55 (2012) 3588-3592.
[53] Y. Otake, K. Izawa, Process for preparation of optically active aminoepoxide derivatives, in, Ajinomoto Co., Inc., Japan . 2003, pp. 30 pp.
[54] L. Roux, C. Charrier, A. Defoin, P. Bisseret, C. Tarnus, Iodine(III)-mediated ring expansion: an efficient and green pathway in the synthesis of a key precursor for the design of aminopeptidase (APN or CD13) inhibitors, Tetrahedron, 66 (2010) 8722-8728.
[55] J. Britton, J.M. Chalker, C.L. Raston, Rapid Vortex Fluidics: Continuous Flow Synthesis of Amides and Local Anesthetic Lidocaine, Chem. - Eur. J., 21 (2015) 10660-10665.
[56] P. Riente, J. Yadav, M.A. Pericàs, A Click Strategy for the Immobilization of MacMillan Organocatalysts onto Polymers and Magnetic Nanoparticles, Org. Lett., 14 (2012) 3668-3671.

List of Captions

Figure 1. IMP-1 inhibitor reported by Liénard *et al*. [40].

Figure 2: Interactions of docked ligand **13a** at the active site of IMP-1 (PDB: 1JJT). Atom colors are as follows: blue–nitrogen, red–oxygen, grey–carbon and yellow-zinc atom (of IMP-1), and red–oxygen, blue–nitrogen, green–carbon, and yellow–sulfur (on inhibitor).

Figure 3. Imipenem-induced growth inhibition of *E. cloacae* ECL1 expressing IMP-4 in the absence of an MBL inhibitor (A, left) and the presence of compound **13a** (B, right).

Figure 4. Imipenem-induced growth inhibition of *E. coli* BL21 expressing IMP-1 in the absence of an MBL inhibitor (A, left) and the presence of compound **13a** (B, right).

Scheme 1. Reagents and conditions: (a) $NaHCO_3$, H_2O, r.t., overnight, 65%; (b) neat, 100 °C, 90 min, 62%; (c) DMF, r.t., 2 h, 59%.

Scheme 2. Reagents and conditions: (a) Boc_2O, NaOH, THF/H_2O, r.t., overnight, 100%; (b) BnBr, NaOMe, MeOH, 40 °C, 3 h, 64%; (c) $SOCl_2$, MeOH, Δ, 3 h, 100%.

Scheme 3. Reagents and conditions: (a) **1a, 1b or 1c**, HBTU, DIPEA, THF, r.t., overnight; (b) NaOH, H_2O/MeOH, r.t., 1 h.

Table 1

Inhibitory activities of the target compounds **13a-c**, **14a-c**, **15a-c**, **16a-c** and **1a** against IMP-1. Benzylpenicillin was used as the substrate.

Table 2

Imipenem MIC values in the absence or presence of compound **13a**

Re: Ms. Ref. No.: EJMECH-D-15-02298 – Revised Manuscript Submission

"Design, synthesis, and *in vitro* and biological evaluation of potent amino acid-derived thiol inhibitors of the metallo-β-lactamase IMP-1" by Arjomandi and co-workers.

Highlights:

- Inhibitors of metallo-β-lactamase were synthesized from aromatic amino acids.
- These compounds were assayed in vitro against IMP-1.
- A decrease in the resistance of MBL-producing bacteria towards imipenem was shown.
- Docking studies were performed on these molecules.