



Synthesis and enzyme-based evaluation of analogues *L*-tyrosine thiol carboxylic acid inhibitor of metallo- β -lactamase IMP-1

Omid Khalili Arjomandi, Mahboubeh Kavousi & Hadi Adibi

To cite this article: Omid Khalili Arjomandi, Mahboubeh Kavousi & Hadi Adibi (2019) Synthesis and enzyme-based evaluation of analogues *L*-tyrosine thiol carboxylic acid inhibitor of metallo- β -lactamase IMP-1, Journal of Enzyme Inhibition and Medicinal Chemistry, 34:1, 1414-1425, DOI: [10.1080/14756366.2019.1651314](https://doi.org/10.1080/14756366.2019.1651314)

To link to this article: <https://doi.org/10.1080/14756366.2019.1651314>



© 2019 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.



View supplementary material [↗](#)



Published online: 11 Aug 2019.



Submit your article to this journal [↗](#)



Article views: 42



View Crossmark data [↗](#)

ORIGINAL ARTICLE



Synthesis and enzyme-based evaluation of analogues *L*-tyrosine thiol carboxylic acid inhibitor of metallo- β -lactamase IMP-1

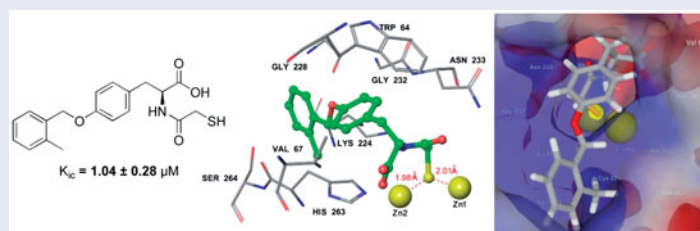
Omid Khalili Arjomandi^{a,b} , Mahboubeh Kavousi^c and Hadi Adibi^a

^aPharmaceutical Sciences Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran; ^bThe University of Queensland, School of Chemistry and Molecular Biosciences, Brisbane, Australia; ^cDepartment of Biochemistry, Pasteur Institute of Iran, Tehran, Iran

ABSTRACT

The emergence of drug-resistant pathogenic bacteria is occurring due to the global overuse and misuse of β -lactam antibiotics. Infections caused by some bacteria which secrete metallo- β -lactamases (enzymes that inactivate β -lactam antibiotics) are increasingly prevalent and have become a major worldwide threat to human health. These bacteria are resistant to β -lactam antibiotics and MBL-inhibitor/ β -lactam antibiotic combination therapy can be a strategy to overcome this problem. So far, no clinically available inhibitors of metallo- β -lactamases (MBLs) have been reported. In this study, *L*-benzyl tyrosine thiol carboxylic acid analogues (**2a–2k**) were synthesized after the study of computational simulation by adding of methyl, chloro, bromo and nitro groups to the benzyl ring for investigation of SAR analysis. Although the synthesized molecules **2a–k** shows the potent inhibitory effects against metallo- β -lactamase (IMP-1) with the range of K_{ic} values of 1.04–4.77 μ M, they are not as potent as the candidate inhibitor.

GRAPHICAL ABSTRACT



ARTICLE HISTORY

Received 5 June 2019
Revised 24 July 2019
Accepted 26 July 2019

KEYWORDS

β -lactam; β -antibiotic resistance; metallo- β -lactamase; inhibitor; computational modelling

Introduction

The β -lactam antibiotics are the most frequently prescribed drugs used in the treatment of bacterial infections since the 1940s. The β -lactam antibiotic resistance crisis has been attributed to the global overuse and misuse of these medications. The study of classifications β -lactam antibiotics and mechanisms of their inactivation by resistant bacteria can assist scientists to design a strategy to overcome this problem. So far, the β -lactamases have been classified to serine- β -lactamases (SBLs, classes A, C and D) and metallo- β -lactamases (MBLs, class B) based on their mechanisms of action. Moreover, MBLs are further divided into three subclasses, B1, B2 and B3, which differ in their amino acid sequences and metal occupancies.^{1–7} A fourth MBL subclass B4 has been tentatively identified as well.^{8–9} What MBLs have in common is having an active site that is mostly occupied by two zinc ions and Table-1 summarise amino acid types chelated into Zinc ions.^{10–18}

In total, all MBLs are characterised by $\alpha\beta\beta\alpha$ quaternary structural fold and zinc ions of the active sites are located at the edge of the $\beta\beta$ sheets at a distance measurement range nearly 3.4–4.4 Å¹⁹ (Figure 1).

In this research, only IMP-1, a subclass B1 enzyme, was only investigated. IMP-1 is secreted by bacteria such as *Pseudomonas aeruginosa* and *Serratia marcescens*. In the active site of this enzyme, Zinc 1 is coordinated by a water molecule and three identical amino acids (His116, His118 and His196) with tetrahedral arrangement while Zinc 2 is coordinated by two water molecules and three different amino acids (Asp120, Cys221 and His263) in distorted trigonal bipyramidal geometry. The proposed mechanism in Figure 2 shows that how the activated water molecule starts the process of hydrolysis of β -lactam antibiotics and convert them to useless drugs.^{20–22}

One strategy to overcome this problem is to find a potent inhibitor that can attach to the active site of IMP-1 and prevent the hydrolysis of the β -lactam rings of antibiotics. Although numerous MBLs inhibitors have been reported in the literature, there is no clinically available inhibitor. Moreover, none of the clinically useful serine- β -lactamases inhibitors, such as clavulanic acid, which is co-administered with penicillin is not effective against MBLs.²³ Therefore, research and discovery of a clinically MBLs inhibitor is still in progress. So far, various inhibitors of MBLs

CONTACT Hadi Adibi hadibi@kums.ac.ir; Omid Khalili Arjomandi omid.arjomandi@uqconnect.edu.au Pharmaceutical Sciences Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran.

© 2019 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

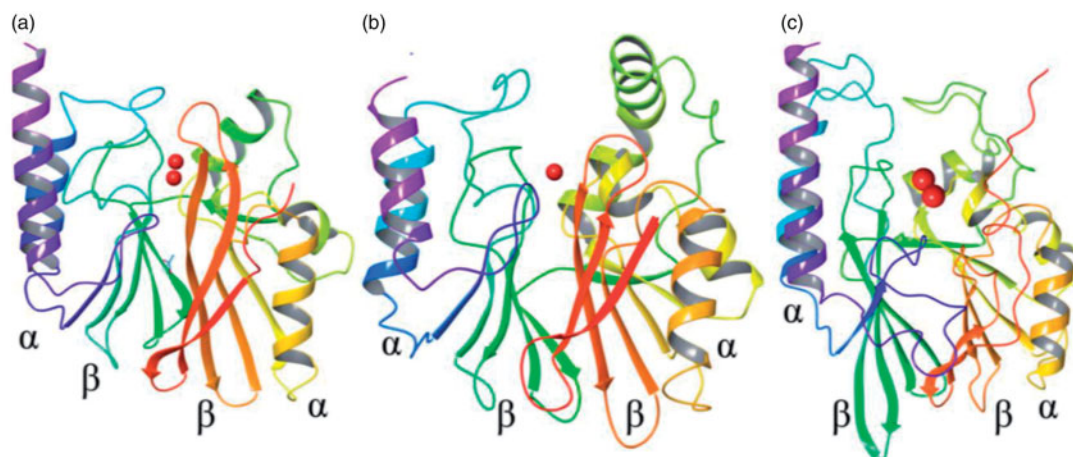


Figure 1. 3D structures representing MBLs. The α -helices are shown in purple and yellow, β -sheets in green and red and Zn^{2+} ions as red spheres. (a) Subclass B1, IMP-1 (PDB code: 1JJT). (b) Subclass B2, CphA (PDB code: 1X8G). (c) Subclass B3, L1 (PDB code: 1SML).

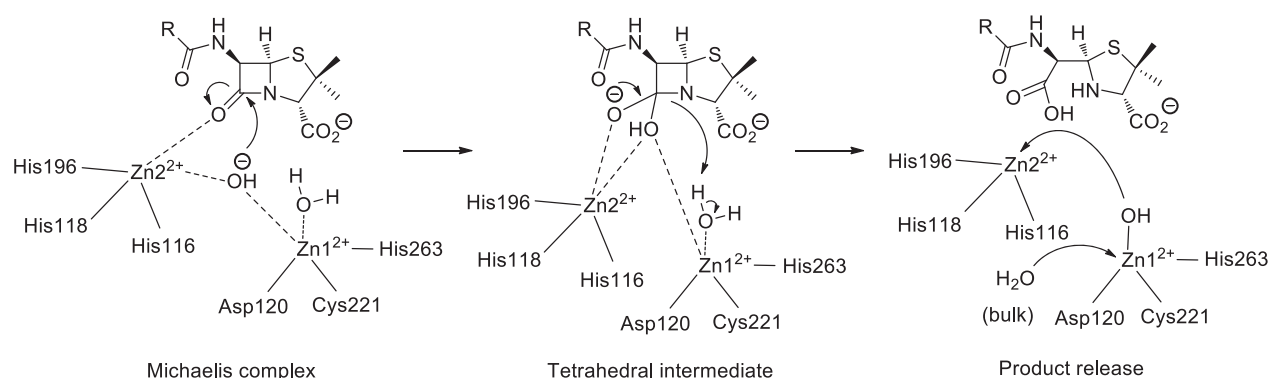


Figure 2. The proposed mechanism of hydrolysis of β -lactam antibiotics by IMP-1.

have been reported in different categories such as thiols,^{24–30} sulphates,³¹ dicarboxylic acids,^{32–37} trifluoromethylketones and alcohols,³⁸ hydroxamates,³⁹ tetrazoles,⁴⁰ sulphonamides and sulphonyl hydrazones.⁴¹ Compounds containing a zinc-binding sulphur atom comprise the first category of MBLs inhibitors.⁷

Thiol carboxylic acid compounds have been reported as the most common inhibitors of IMP-1.⁷ For instance, thiomandelic acid and 2-mercapto-5-phenylpentanoic acid were discovered as a broad-spectrum inhibitor for MBLs where Cd-NMR studies demonstrated that the sulphur atom of the compound intercalates the metal ions within the active site and displaces the bridging water molecule that is essential for the MBL hydrolase activity. Further potent thiol containing compound reported with good IC₅₀ by Büttner et al.⁴² The other potent thiol carboxylic acid compounds, bisthiazolidines, reported by González et al.⁴³ with K_i values in a range of 7–19 μM against NDM-1. The co-crystallised enzyme/inhibitors of these compounds demonstrated that the sulphur atom of the mercaptomethyl group is positioned nearly equidistant between the two zinc ions, displacing the bridging water/hydroxide molecule that is proposed to be the attacking nucleophile, but with little impact on the Zn1–Zn2 distance.⁴³ Further bisthiazolidines derivatives reported by Hinchliffe et al.⁴⁴ with a broader range of assay on MBLs. The result of inhibition study is fascinating as these molecules capable of inhibition of all three subclasses with a highest K_i value of 41 μM against GOB-18.⁴⁴ These compounds were very potent against IMP-14 with K_i values in a range of 6–14 μM . Arjomandi et al.¹ also demonstrated L-benzyl tyrosine thiol carboxylic acid (Figure 3) as a potent MBL inhibitor with the K_{ic} value of 86 nM against IMP-1. In this study, this

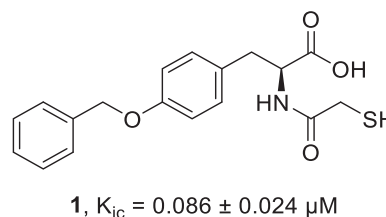


Figure 3. The chemical structure and K_{ic} value of inhibitor **1** against IMP-1.

inhibitor was used as a candidate molecule for further modification and development with the aim of improving inhibition activity.

Various modifications on the metal chelating inhibitor **1** can be exerted and investigated for the aim the study of improving inhibition activity. The study of computational modelling predicts the thiol group of the candidate inhibitor is coordinated to both Zinc ions of the active site of the enzyme which is probably one of the key interactions for strong chelating. Therefore, the substitution of the carboxylic acid group with a thiol moiety may improve the potency of the inhibitory activity as there will be two thiol groups for metal chelating. On the other hand, computational simulation is only a predicted model and does not reveal the real configuration of the ligand and its interactions with the amino acids of the active site, thus, the replacement of the thiol group with the carboxylic acid can also be investigated when the modified molecule would have two carboxylic acid groups. The alternative modification is the substitution of hydrogen(s) of the methylene groups of the skeleton with the electron-donating and electron-

withdrawing groups or hydrogen acceptor and donor moieties and examination of their interactions with the amino acids of the active site for the study of a better fixing in the active site. The subsequent modification is the substituting of the central phenyl ring with heteroaromatic rings which may lead to a better fixing of the molecule into the active site due to the creation of further interactions like hydrogen bond(s). Moreover, removing either one or two methylene groups of the skeleton reduce the size of the molecule that may create a better conformation and configuration with the creation of stronger interactions with Zinc ions or amino acids of the active site. The other modification is the study of addition of hydrogen acceptor and donor groups or electron withdrawing or electron donating moieties on either central phenyl ring or the tale benzyl ring. In this study, the latest modification was investigated by the addition of methyl, chloro, bromo and nitro groups to the benzyl ring of the candidate molecule (Figure 4) and *in silico* modelling was used for the prediction of ligand binding into the active site of IMP-1.

Computational modelling

For the study of computational modelling, the crystal structure of IMP-1 enzyme (PDB code: 1JJT) was downloaded from Protein Data Bank (PDB) and Chain B was deleted as the topologies of the

- 2a, R= 2-methyl
 2b, R= 3-methyl
 2c, R= 4-methyl
 2d, R= 2-chloro
 2e, R= 3-chloro
 2f, R= 4-chloro
 2g, R= 2-nitro
 2h, R= 3-nitro
 2i, R= 4-nitro
 2j, R= 3-bromo
 2k, R= 4-bromo

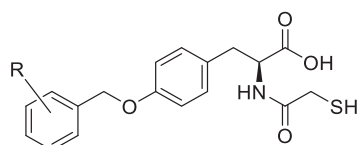


Figure 4. The chemical structures of analogues *L*-thiol carboxylic acid.

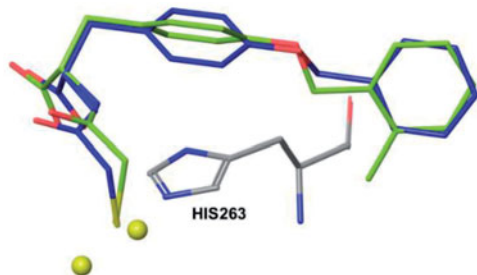


Figure 5. The typical superimposed of docked inhibitor 2a (green) and the reference inhibitor 1 (blue) into the active site of IMP-1.

active sites of both chains A and B are identical. Further elimination for chain A was carried out by removing water molecules (solvents), single Zinc ion and the coordinated ligand of the active site. The cavity of the active site with two zinc ions was set by MolDock for the investigation of docking study. Compounds 2a–2k and inhibitor 1 as the reference molecule were docked into the active site of IMP-1 and the study of their coordination indicates that they almost have similar configurations and conformation in the active site with slightly rotation of the central phenyl ring but are still flanked between Trp64 and His263 which may create π – π stacking interactions (Figure 5). The calculated docking energies (E_{score}) of all analogues were higher and better than the lead compound. On the other hand, the predicted distance of the coordination of the thiol group of most analogues to Zinc 2 is shorter than Inhibitor 1 which can be considered as one of the key factors for stronger chelation. The predicted distance between Zinc 1 and the thiol group of target molecules and the reference ligand is nearly unaffected (Table 1). The docking also anticipates the creation of hydrogen bond between carboxylic acid group and HIS263 for all molecules including the reference compound (Figure 6). Altogether and based on the energy of the computational modelling of analogues and prediction of stronger binding to one of Zinc ions, the molecules were synthesized and their *in vitro* inhibitions were measured against IMP-1.

Enzyme kinetics studies

The IMP-1 enzyme, lacking the first 21 signal peptide amino acid residues, was expressed and purified using the protocol of Vella et al.^{45–46} The reported methods for monitoring of MBLs activities

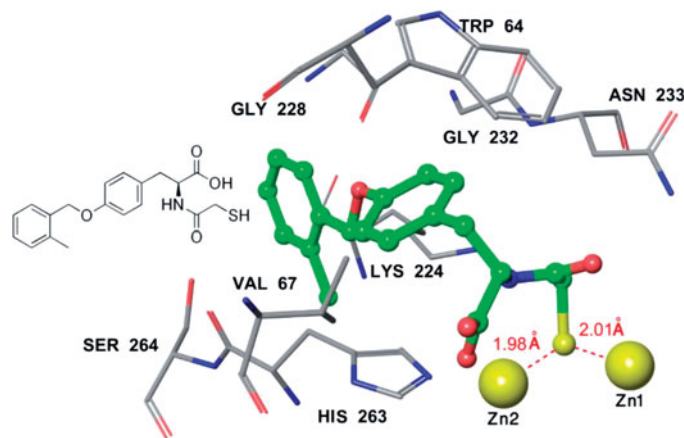


Figure 6. Interaction of pose 2a at the active site of IMP-1 (PDB: 1JJT). Atom colours are as follows: blue – nitrogen, red – oxygen, grey – carbon and yellow – zinc atom (of IMP-1), and red – oxygen, blue – nitrogen, green – carbon, and yellow – sulphur (on inhibitor).

Table 1. Subclasses of MBLs and their active site amino acids that chelated Zinc ions.

Subclass	Examples	Zinc–amino acid ligands		Involvement of Zn2+ ions for catalytic activity
		Zn1 ligands	Zn2 ligands	
B1	IMP-1, CcrA, VIM, NDM-1, BclI	His 116 His 118 His 196	Asp 120 Cys 221 His 263	Mainly di-zinc but BclI is active with either one or two Zn ²⁺ ions
B2	CphA, Imis	Asn 116 His 118 His 196	Asp 120 Cys 221 His 263	Mono-zinc (Zn2 site) but binding of Zn1 inhibits the MBL
B3	L1 (tetramer), FEZ-1, GOB-18	His/Gln 116 His 118 His 196	Asp 120 His 121 His 263	Di-zinc

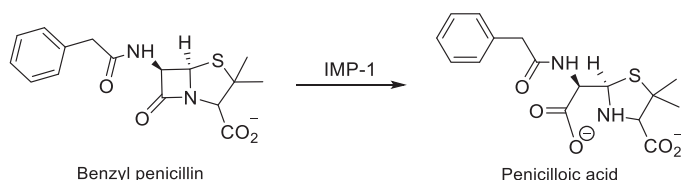


Figure 7. Inactivation of benzyl penicillin by MBLs.

are based on the consequences of the hydrolysis of β -lactam ring of substrates. The MBL-catalysed reaction is usually monitored by following the changing absorbance at the wavelength where the difference in the molar absorptivity, $\Delta\epsilon$, is maximal or near the maximum value. There are a few β -lactam substrates which can be used for *in vitro* assays of MBLs inhibitors. CENTA,⁴⁷ a type of cephalosporin, and Penicillin G (benzyl penicillin)⁴⁸ are well-known substrates due to commercial availability, inexpensive prices and comfort of their syntheses. For this study, Penicillin G was used as CENTA was not compatible with the inhibitor type as reported by Arjomandi et al.¹ The monitoring of hydrolysis of benzyl penicillin by the synthesized compounds was carried out in UV plate at wavelength 235 nm as no chromophore is released (Figure 7). The concentration of benzyl penicillin is reduced upon hydrolysis by IMP-1 causing a decrease in the absorption at 235 nm over the time, thereby allowing a way to monitor the reaction. Inhibitors of metallo- β -lactamase decrease the rate of hydrolysis of benzyl penicillin, thus, providing a way of measuring the activities of inhibitors against MBLs.

Benzyl penicillin solutions were prepared fresh at the time of assay as their activities will change and stop after 24 h even with keeping in the fridge.¹ In the first step, the inhibitory activities (IC_{50} values) of the inhibitors **2a–k** were measured by a screening assay which evaluated the residual activity of the enzyme. The assay results showed that all compounds **2a–k** exhibited approximately 50% inhibition at the range of concentration of 1–5 μ M. Further kinetic analyses then allowed the determination of K_{ic} values of these compounds based on the procedure reported by Arjomandi et al.¹ Inhibition assays for IMP-1 were performed in 96 well 400 μ L UV multi-titre plates using a UV multi-plate spectrophotometer. The hydrolysis rate of benzyl penicillin (penicillin G sodium salt) was measured at $\lambda = 235$ nm.⁴⁹ The initial screening assay (IC_{50} values) was performed in duplicate, with benzyl penicillin as the substrate and HEPESX (50 mM HEPES, 0.1 M NaCl, 100 mM $ZnCl_2$, pH 7.0) as the buffer at 25 °C. The final concentrations of enzyme and benzyl penicillin were 10 nM and 500 μ M, respectively. Bovine serum albumin (a final concentration of 20 μ g/mL) was added to the enzyme for stability. For each well, 196 μ L substrate and 4 μ L inhibitor were added followed by the addition of 200 μ L of enzyme. The rate of the hydrolysis of substrate was recorded for 5 min. To measure K_{ic} value, four solutions with different concentrations for each molecule were prepared and then were assayed based on the aforementioned procedure while the concentrations of substrate in wells were 200, 400, 600, 800 and 1000 μ M. The raw kinetic data were analysed by non-linear regression using the Win Curve Fit program (Kevin Raner Software) and the K_{ic} value was calculated for each molecule.¹ The K_{ic} values of molecules **2a–k** are in the range of 1.04–4.77 μ M which was unexpected based on the computational modelling results. Although the introduced compounds are still potent enzyme-based inhibitors, they are nearly at least ten times less as potent as the lead inhibitor is. Due to the less inhibition activities of the synthesized molecules in compare to the reference compound, their cell-based assays have not carried out.

Table 2. The computational modelling results and K_{ic} values of inhibitor 1 and compounds **2a–k**.

Compound	MolDock score (kcal/mol)	Distance (Å) between Zn1 ion and SH	Distance (Å) between Zn2 ion and SH	K_{ic} (μ M)
1	−190.9	1.93	1.94	0.086 ± 0.024
2a	−201.1	2.04	1.85	1.04 ± 0.28
2b	−202.9	1.93	1.96	1.15 ± 0.49
2c	−194.1	1.94	1.90	1.07 ± 0.21
2d	−197.3	2.04	1.85	1.81 ± 0.90
2e	−194.2	1.94	1.90	4.77 ± 2.81
2f	−195.5	2.03	1.88	3.20 ± 0.59
2g	−201.9	1.94	1.91	1.65 ± 0.34
2h	−199.8	1.94	1.90	2.86 ± 1.23
2i	−207.0	1.94	1.90	3.20 ± 0.62
2j	−199.5	1.93	1.95	3.14 ± 0.71
2k	−194.1	1.93	1.94	3.30 ± 0.90

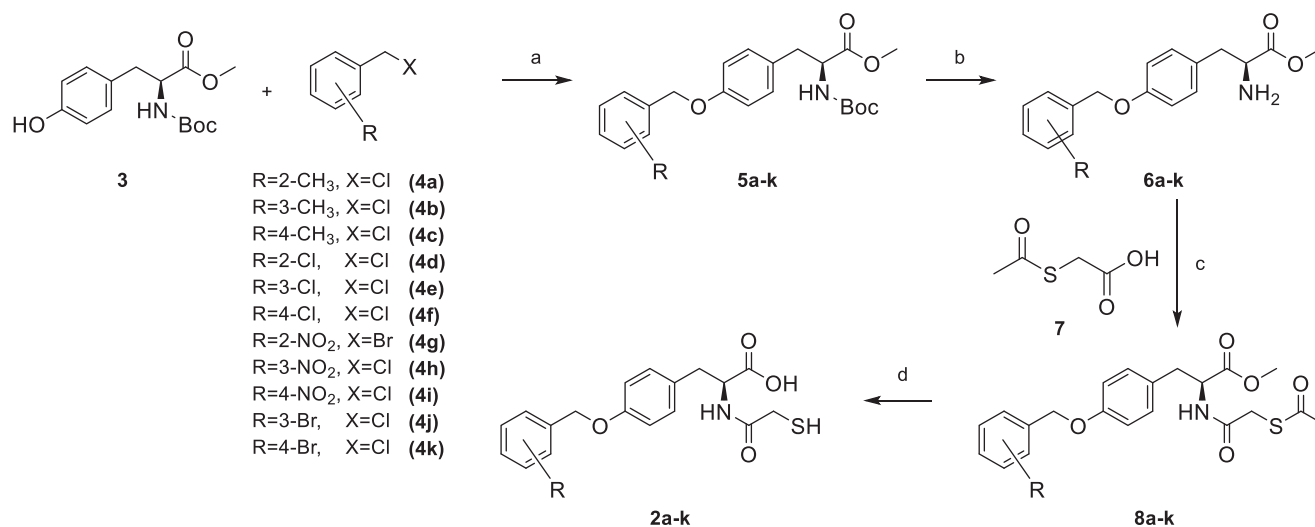
Among the reported molecules, compounds with methyl groups **2a–c** are better enzyme-based inhibitors when compared compounds **2d–k**. In particular *ortho* and *para* substituted compounds **2a** and **2c** are similar and better than *meta*-substituted compound **2b** which indicates the influence of electron donating group. Although the docking poses failed to show any interaction of the benzyl group in the receptor site, apparently a monosubstituted electron donating group on benzyl ring can lead to a better and stronger affinity of the inhibitor in the active site while electron withdrawing one decrease that affinity.

The achieved experimental results indicate that all compounds **2a–k** are potent inhibitors against IMP-1 and the *ortho*-substituted compounds on benzyl ring show the highest inhibitory activities than *meta*- and *para*-substituted ones (Table 2).

To compare the information of the computational modelling and the achieved experimental data, the K_{ic} values were compared with the predicted calculated energy and predicted distances of Zinc ions (Table 2). Although all compounds **2a–k** were potent inhibitors against IMP-1, the trend of comparison of theoretical energy did not match with the activities of inhibitors. Therefore, adding substituents to any position of the benzyl ring of the reference compound **1** apparently leads to a decrease in their inhibitory activities which can be described based on the restricted rotation of the benzyl group of the inhibitor(s). Consequently, the addition of either electron donor or electron withdrawing group to the benzyl ring can decrease the potency of inhibitors against IMP-1.

Chemistry

The synthesis pathway of compounds **2a–2k** was similar to what had been reported by Arjomandi et al.¹ However, the purchased Boc-protected *L*-tyrosine methyl ester **3** was used as the precursor to reduce the number of steps in the synthesis (Scheme 1). Compounds **5a–k** were synthesized by Williamson ether synthesis procedure with refluxing a mixture of Boc *L*-tyrosine methyl ester **3**, compounds **4a–k** and potassium carbonate in acetone with the isolated yields over 80%.⁵⁰ The BOC group then was removed by adding compounds **5a–k** into the mixture of TFA in chloroform to produce compounds **6a–k** with the isolated yields of 98%.⁵¹ Compound **7** (acetylthio acetic acid) was synthesized by the procedure that was reported by Arjomandi et al.¹ The amidation of *L*-amino acid methyl esters **6a–k** and acetylthio acetic acid **7** was carried out by HBTU as a coupling reagent and DIPEA as a base to produce compounds **8a–k** which then purified by flash column chromatography.⁵² One of the by-products of HBTU is tetramethyl urea which is formed in the reaction and its ¹H NMR signal



Scheme 1 Reagents and conditions: (a) Acetone, K₂CO₃, Δ, 24 h; (b) TFA, CHCl₃, r.t, overnight, 98%; (c) Compound **7**, HBTU, DIPEA, THF, r.t, 24 h; (d) NaOH, H₂O/MeOH, r.t, 1 h, under nitrogen.

appears at 2.7 ppm. To remove this impurity, running a mixture of 30–70% petroleum ether–ethyl acetate solvent in flash column chromatography can take out tetra methyl urea as well as other impurities. The monitoring of the fraction samples by TLC indicated that no product(s) is available. The final products then were collected with increase the percentage of the polar component of the solvent. Intermediates **8a–k** are new compounds, and their ¹H NMR and ¹³C-NMR spectra (as observed by the appearance of the characteristic thioacetyl methyl group) as well as LRMS and HRMS (positive mode) were recorded and confirmed the constitutions of molecules. The final hydrolysed compounds **2a–k** were prepared by the treatment of intermediates **8a–k** with sodium hydroxide solution under nitrogen to give the final thiol carboxylic acid products (Scheme 1). This is extremely important for the final hydrolysis reaction to be carried out under nitrogen as thiol carboxylic acids are sensitive to aerial oxidation. In addition, the isolated compounds **2a–k** need to be kept in the sealed container as well. Compounds **2a–k** are new and ¹H NMR and ¹³C-NMR spectra (by evaluating of the disappearance of acetyl and methyl groups and the appearance of SH and COOH groups), as well as LRMS and HRMS (negative mode), were recorded and confirmed the constitutions of molecules.

Conclusions

We described a straightforward and efficient synthesis of analogues of *L*-benzyl tyrosine thiol carboxylic acid **1**. These compounds demonstrated potent enzyme-based *in vitro* inhibitory activities against IMP-1 with *K_i* values of ranging 1.04 ± 0.28–4.77 ± 2.81 μM but they were less potent than the lead compound. The compounds with electron donating group to *ortho*, *meta* and *para* of the benzyl ring of candidate molecule show better inhibition than electron withdrawing one. The addition of substitution to the benzyl ring of the potent cell-based inhibitor **1** decreases its activity against IMP-1.

Experimental

Compound characterisation. General information

All chemicals were purchased from Sigma-Aldrich (Chicago, IL), Merck (Kenilworth, NJ) and Fluka Chemical Companies (Milwaukee,

WI). All NMR experiments were recorded on Bruker AVANCE 500, 400 or 300 MHz spectrometers (Billerica, MA). Chemical shifts are reported in parts per million (ppm) on a δ scale and referenced to the residual solvent peak (¹H, 7.24 ppm, ¹³C, 77.0 ppm for CDCl₃; ¹H, 2.49 ppm, ¹³C, 39.5 ppm for DMSO-*d*₆; ¹H, 3.30 ppm, ¹³C, 49.5 ppm for CD₃OD). 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 the positive and the negative ionisation mode. LR-ESI were recorded on a Bruker HCT 3D Ion Trap and HR-ESI were performed on a Bruker MicroTOF-Q with the DIONEX Ultimate 3000 LC in the positive and the negative electrospray ionisation mode, with CH₃OH as a solvent. For IR analyses, samples were measured neat on a PerkinElmer Frontier FT-IR spectrometer (PerkinElmer, Waltham, MA).

Synthesis

General procedure 1: Williamson ether synthesis

The general procedure reported by Wolf et al.⁵⁰ was used for the synthesis of compounds **5a–k**. A mixture of *N*-Boc-*L*-tyrosine methyl ester (2.0 g, 6.8 mmol), benzyl derivative **4a–k** (13.5 mmol), potassium carbonate (1.87 g, 13.5 mmol) and sodium iodide (100 mg as the catalytic amount) in acetone (50 mL) was heated under reflux, under nitrogen, for 24 h. The resulting mixture was then filtrated and evaporated under the vacuum to give the crude oil which was purified by flash chromatography (30–100% EtOAc in petroleum ether).

General procedure 2: Boc deprotection

The general procedure reported by Benthem et al.⁵¹ was used for the synthesis of compounds **6a–k**. A mixture of *L*-Boc protected compounds **5a–k** (5 mmol), chloroform (30 mL) and TFA (1.14 g, 0.76 mL, 10 mmol) was stirred at room temperature, and the progress of the reaction was monitored by TLC. Then the organic solution was washed with saturated sodium bicarbonate (30 mL), dried over sodium sulphate and evaporated under the vacuum to give the products **6a–k** which was used for the next step without further purification.

General procedure 2: Amide coupling reactions

The general method reported by Knorr et al.⁵² was used for the synthesis of compounds **8a–k**. A mixture of deprotected Boc compounds **6a–k** (4.0 mmol), 2-(acetylthio)acetic acid **7** (0.59 g, 4.4 mmol), HBTU (1.67 g, 4.4 mmol) and DIPEA (1.56 g, 2.10 mL, 12.00 mmol) in THF (30 mL) was stirred at room temperature for 24 h. Then the organic solvent was evaporated under the vacuum, and the residue was dissolved in DCM (50 mL). The organic phase was then washed with saturated sodium bicarbonate (2 × 50 mL) and evaporated under the vacuum to give the crude oil which was purified by flash chromatography (30–100% EtOAc in petroleum ether) to give the pure products **8a–k**.

General procedure 3: Saponification

Compounds **2a–k** were synthesized using the following general procedure: compounds **8a–k** (3.0 mmol) were dissolved in methanol (10 mL), then sodium hydroxide solution (1 M, 10 mL) was added and the solution was stirred under nitrogen for an hour. The organic solvent (methanol) was evaporated under the vacuum, then water (50 mL) was added and the combined water phase was washed with ethyl acetate (50 mL) and DCM (50 mL) under nitrogen. Then pH was adjusted to 1 using concentrated HCl. The product in the water phase was extracted with ethyl acetate (50 mL) and washed with water (5 × 50 mL)¹ to remove acetic acid. The organic phase was dried over sodium sulphate, filtered and evaporated under the vacuum to give the pure product **2a–k**. All ¹H NMR, ¹³C NMR, LRMS and HRMS (negative mode) spectra were recorded.

Analytical information of synthesized molecules

(S)-Methyl 2-((tert-butoxycarbonyl)amino)-3-(4-((2-methylbenzyl)oxy)phenyl)propanoate (5a). Pale yellow viscous oil, 2.17 g (80%). ¹H NMR (500 MHz, CDCl₃): δ 7.34 (1H, d, *J* = 7.1 Hz, Ar-H), 7.26–7.16 (3H, m, Ar-H), 7.03 (2H, d, *J* = 8.5 Hz, Ar-H), 6.89 (2H, d, *J* = 8.5 Hz, Ar-H), 4.98 (2H, s, CH₂O), 4.96 (1H, d-broad, *J* = 7.5 Hz, NH), 4.54 (1H, q, *J* = 6.9 Hz, CH₂CHNH), 3.70 (3H, s, CH₃O), 3.04 (1H, dd, *J* = 13.8, 5.8 Hz, CH_AH_BCHNH), 2.99 (1H, dd, *J* = 13.8, 5.8 Hz, CH_AH_BCHNH), 2.35 (3H, s, CH₃Ph), 1.40 (9H, s, C(CH₃)₃). ¹³C NMR (125 MHz, CDCl₃): δ 172.4 (CO₂CH₃), 158.0 (CO₂Boc), 155.1 (Ar-C), 136.7 (Ar-C), 134.7 (Ar-C), 130.4 (Ar-C), 130.2 (Ar-C), 128.6 (Ar-C), 128.2 (Ar-C), 128.2 (Ar-C), 126.0 (Ar-C), 114.8 (Ar-C), 79.9 (C(CH₃)₃), 68.6 (CH₂O), 54.5 (CH₂CHNH), 52.1 (CH₃OCO), 37.5 (CH₂CHNH), 28.3 (C(CH₃)₃), 18.8 (CH₃Ph). HRMS calculated for C₂₃H₂₉NNaO₅ [M + Na]⁺ 422.1938, found 422.1939.

(S)-Methyl 2-amino-3-(4-((2-methylbenzyl)oxy)phenyl)propanoate (6a). Orange solid, 1.47 g (98%). ¹H NMR (500 MHz, CDCl₃): δ 7.39 (1H, d, *J* = 7.1 Hz, Ar-H), 7.27–7.17 (3H, m, Ar-H), 7.11 (2H, d, *J* = 8.5 Hz, Ar-H), 6.93 (2H, d, *J* = 8.5 Hz, Ar-H), 5.00 (2H, s, CH₂O), 3.71 (3H, s, CH₃O), 3.70 (1H, t, *J* = 6.6 Hz, CH₂CHNH), 3.04 (1H, dd, *J* = 13.8, 5.2 Hz, CH_AH_BCHNH), 2.84 (1H, dd, *J* = 13.8, 7.7 Hz, CH_AH_BCHNH), 2.36 (3H, s, CH₃Ph), 1.97 (2H, bs, NH₂). ¹³C NMR (125 MHz, CDCl₃): δ 175.3 (CO), 157.9 (Ar-C), 136.6 (Ar-C), 134.7 (Ar-C), 130.3 (Ar-C), 130.3 (Ar-C), 129.2 (Ar-C), 128.6 (Ar-C), 128.2 (Ar-C), 126.0 (Ar-C), 114.8 (Ar-C), 68.6 (CH₂O), 55.8 (CH₂CHNH), 52.0 (CH₃O), 39.9 (CH₂CHNH), 18.8 (CH₃Ph). HRMS calculated for C₁₈H₂₂NO₃ [M + H]⁺ 300.1594, found 300.1595.

(S)-Methyl 2-(2-(acetylthio)acetamido)-3-(4-((2-methylbenzyl)oxy)phenyl)propanoate (8a). Pale yellow viscous oil, 1.33 g (80%). ¹H NMR (400 MHz, CDCl₃): δ 7.37 (1H, d, *J* = 7.1 Hz, Ar-H), 7.27–7.17

(3H, m, Ar-H), 6.99 (2H, d, *J* = 8.5 Hz, Ar-H), 6.88 (2H, d, *J* = 8.5 Hz, Ar-H), 6.60 (1H, d, *J* = 7.7 Hz, NH), 4.98 (2H, s, CH₂O), 4.77 (1H, dt, *J* = 7.7, 5.8 Hz, CH₂CHNH), 3.71 (3H, s, CH₃O), 3.52 (2H, dd, *J* = 25.0, 15.0 Hz, CH₂S), 3.08 (1H, dd, *J* = 13.8, 5.5 Hz, CH_AH_BCHNH), 3.00 (1H, dd, *J* = 13.8, 6.1 Hz, CH_AH_BCHNH), 2.35 (3H, s, CH₃CO), 2.32 (3H, s, CH₃Ph). ¹³C NMR (100 MHz, CDCl₃): δ 195.2 (COS), 171.6 (CO₂), 167.6 (CONH), 158.0 (Ar-C), 136.6 (Ar-C), 134.7 (Ar-C), 130.4 (Ar-C), 130.3 (Ar-C), 128.6 (Ar-C), 128.3 (Ar-C), 127.8 (Ar-C), 126.0 (Ar-C), 114.8 (Ar-C), 68.5 (CH₂O), 53.5 (CH₂CHNH), 52.3 (CH₃O), 36.7 (CH₂CHNH), 32.7 (CH₂S), 30.1 (CH₃CO), 18.8 (CH₃Ph). HRMS calculated for C₂₂H₂₅NNaO₅ [M + Na]⁺ 438.1346, found 438.1349.

(S)-2-(2-Mercaptoacetamido)-3-(4-((2-methylbenzyl)oxy)phenyl)propanoic acid (2a). Off white solid, 0.66 g (61%). ¹H NMR (400 MHz, CD₃OD): δ 7.34 (1H, d, *J* = 7.3 Hz, Ar-H), 7.24–7.10 (5H, m, Ar-H), 6.99 (2H, d, *J* = 8.5 Hz, Ar-H), 5.00 (2H, s, ArCH₂O), 4.62 (1H, dd, *J* = 8.3, 5.3 Hz, CH₂CHNH), 3.14 (1H, dd, *J* = 14.0, 6.0), 3.13 (2H, s, CH₂SH), 2.95 (1H, dd, *J* = 14.0, 8.2 Hz, CH_AH_BCHNH), 2.33 (3H, s, CH₃). ¹³C NMR (100 MHz, CD₃OD): δ 174.4 (CO₂H), 172.9 (CONH), 159.3 (Ar-C), 138.0 (Ar-C), 136.4 (Ar-C), 131.5 (Ar-C), 131.3 (Ar-C), 130.3 (Ar-C), 129.7 (Ar-C), 129.2 (Ar-C), 126.9 (Ar-C), 115.8 (Ar-C), 69.7 (PhCH₂O), 55.3 (CH₂CHNH), 37.5 (CH₂CHNH), 28.0 (CH₂SH), 18.9 (CH₃). HRMS calculated for C₁₉H₂₀NO₄S [M-H]⁻ 358.1119, found 358.1125.

(S)-Methyl 2-((tert-butoxycarbonyl)amino)-3-(4-((3-methylbenzyl)oxy)phenyl)propanoate (5b). Pale yellow viscous oil, 2.17 g (80%). ¹H NMR (500 MHz, CDCl₃): δ 7.28–7.17 (3H, m, Ar-H), 7.12 (1H, d, *J* = 7.4 Hz, Ar-H), 7.02 (2H, d, *J* = 8.5 Hz, Ar-H), 6.88 (2H, d, *J* = 8.5 Hz, Ar-H), 4.98 (2H, s, CH₂O), 4.94 (1H, d, *J* = 7.5 Hz, NH), 4.33 (1H, q, *J* = 6.9 Hz, CH₂CHNH), 3.69 (3H, s, CH₃O), 3.07–2.93 (2H, m, CH₂CHNH), 2.35 (3H, s, CH₃Ph), 1.40 (9H, s, C(CH₃)₃). ¹³C NMR (125 MHz, CDCl₃): δ 172.4 (CO₂CH₃), 158.0 (CO₂Boc), 155.1 (Ar-C), 138.3 (Ar-C), 136.9 (Ar-C), 130.3 (Ar-C), 128.7 (Ar-C), 128.5 (Ar-C), 128.2 (Ar-C), 128.2 (Ar-C), 124.6 (Ar-C), 114.9 (Ar-C), 79.9 (C(CH₃)₃), 70.1 (CH₂O), 54.5 (CH₂CHNH), 52.2 (CH₃OCO), 37.5 (CH₂CHNH), 28.3 (C(CH₃)₃), 21.4 (CH₃Ph). HRMS calculated for C₂₃H₂₉NNaO₅ [M + Na]⁺ 422.1938, found 422.1935.

(S)-Methyl 2-amino-3-(4-((3-methylbenzyl)oxy)phenyl)propanoate (6b). Olive green solid, 1.47 g (98%). ¹H NMR (500 MHz, CDCl₃): δ 7.91 (2H, bs, NH₂), 7.24 (1H, t, *J* = 7.6 Hz, Ar-H), 7.20 (1H, s, Ar-H), 7.17 (1H, d, *J* = 7.6 Hz, Ar-H), 7.11 (1H, d, *J* = 7.4 Hz, Ar-H), 7.07 (2H, d, *J* = 8.5 Hz, Ar-H), 6.91 (2H, d, *J* = 8.5 Hz, Ar-H), 4.94 (2H, s, CH₂O), 4.23 (1H, dd, *J* = 7.7, 5.2 Hz, CH₂CHNH), 3.73 (3H, s, CH₃O), 3.23 (1H, dd, *J* = 13.8, 5.2 Hz, CH_AH_BCHNH), 3.13 (1H, dd, *J* = 13.8, 7.7 Hz, CH_AH_BCHNH), 2.34 (3H, s, CH₃Ph). ¹³C NMR (125 MHz, CDCl₃): δ 169.2 (CO), 158.8 (Ar-C), 138.3 (Ar-C), 136.5 (Ar-C), 130.3 (Ar-C), 128.9 (Ar-C), 128.5 (Ar-C), 128.3 (Ar-C), 124.6 (Ar-C), 124.6 (Ar-C), 115.6 (Ar-C), 70.1 (CH₂O), 54.5 (CH₂CHNH), 53.4 (CH₃O), 35.2 (CH₂CHNH), 21.4 (CH₃Ph). HRMS calculated for C₁₈H₂₂NO₃ [M + H]⁺ 300.1594, found 300.1592.

(S)-Methyl 2-(2-(acetylthio)acetamido)-3-(4-((3-methylbenzyl)oxy)phenyl)propanoate (8b). Viscous yellow oil, 1.33 g (80%). ¹H NMR (500 MHz, CDCl₃): δ 7.29–7.16 (3H, m, Ar-H), 7.12 (1H, d, *J* = 7.4 Hz, Ar-H), 6.98 (2H, d, *J* = 8.5 Hz, Ar-H), 6.86 (2H, d, *J* = 8.5 Hz, Ar-H), 6.62 (1H, bs, NH), 4.97 (2H, s, CH₂O), 4.75 (1H, q, *J* = 6.5 Hz, CH₂CHNH), 3.71 (3H, s, CH₃O), 3.52 (2H, dd, *J* = 32.0, 15.1 Hz, CH₂S), 3.07 (1H, dd, *J* = 13.8, 5.5 Hz, CH_AH_BCHNH), 2.99 (1H, dd, *J* = 13.8, 6.1 Hz, CH_AH_BCHNH), 2.35 (3H, s, CH₃CO), 2.31 (3H, s, CH₃Ph). ¹³C NMR (125 MHz, CDCl₃): δ 195.2 (COS), 171.6 (CO₂),

167.7 (CONH), 158.0 (Ar-C), 138.3 (Ar-C), 136.8 (Ar-C), 130.3 (Ar-C), 128.7 (Ar-C), 128.5 (Ar-C), 128.2 (Ar-C), 127.7 (Ar-C), 124.6 (Ar-C), 114.9 (Ar-C), 70.0 (CH₂O), 53.5 (CH₂CHNH), 52.4 (CH₃O), 36.7 (CH₂CHNH), 32.7 (CH₂S), 30.1 (CH₃CO), 21.4 (CH₃Ph). HRMS calculated for C₂₂H₂₅NNaO₅S [M + Na]⁺ 438.1346, found 438.1343.

(S)-2-(2-Mercaptoacetamido)-3-(4-((3-methylbenzyl)oxy)phenyl)propanoic acid (2b). Off white solid, 0.70 g (65%). ¹H NMR (400 MHz, CD₃OD): δ 7.25–7.16 (3H, m, Ar-H), 7.15–7.08 (3H, m, Ar-H), 6.89 (2H, d, *J* = 8.5 Hz, Ar-H), 4.99 (2H, s, ArCH₂O), 4.61 (1H, dd, *J* = 8.3, 5.3 Hz, CH₂CHNH), 3.14 (1H, dd, *J* = 14.0, 6.0, CH_AH_BCHNH), 3.12 (2H, s, CH₂SH), 2.93 (1H, dd, *J* = 14.0, 8.2 Hz, CH_AH_BCHNH), 2.33 (3H, s, CH₃). ¹³C NMR (100 MHz, CD₃OD): δ 174.4 (CO₂H), 172.9 (CONH), 159.3 (Ar-C), 139.3 (Ar-C), 138.7 (Ar-C), 131.4 (Ar-C), 130.3 (Ar-C), 129.5 (Ar-C), 129.4 (Ar-C), 129.1 (Ar-C), 125.6 (Ar-C), 115.9 (Ar-C), 71.0 (PhCH₂O), 55.3 (CH₂CHNH), 37.5 (CH₂CHNH), 28.0 (CH₂SH), 21.4 (CH₃). HRMS calculated for C₁₉H₂₀NO₄S [M-H]⁻ 358.1119, found 358.1129.

(S)-Methyl 2-((tert-butoxycarbonyl)amino)-3-(4-((4-methylbenzyl)oxy)phenyl)propanoate (5c). Off white solid, 2.44 g (90%). ¹H NMR (400 MHz, CDCl₃): δ 7.29 (2H, d, *J* = 7.9 Hz, Ar-H), 7.17 (2H, d, *J* = 7.9 Hz, Ar-H), 7.01 (2H, d, *J* = 8.5 Hz, Ar-H), 6.87 (2H, d, *J* = 8.5 Hz, Ar-H), 4.97 (2H, s, CH₂O), 4.94 (1H, bs, NH), 4.61–4.44 (1H, m, CH₂CHNH), 3.68 (3H, s, CH₃O), 3.02–2.92 (2H, m, CH₂CHNH), 2.34 (3H, s, CH₃Ph), 1.40 (9H, s, C(CH₃)₃). ¹³C NMR (100 MHz, CDCl₃): δ 172.4 (CO₂CH₃), 158.0 (CO₂Boc), 155.1 (Ar-C), 137.7 (Ar-C), 133.9 (Ar-C), 130.3 (Ar-C), 129.3 (Ar-C), 128.1 (Ar-C), 127.6 (Ar-C), 114.9 (Ar-C), 79.9 (C(CH₃)₃), 69.9 (CH₂O), 54.5 (CH₂CHNH), 52.2 (CH₃OCO), 37.5 (CH₂CHNH), 28.3 (C(CH₃)₃), 21.2 (CH₃Ph). HRMS calculated for C₂₃H₂₉NNaO₅ [M + Na]⁺ 422.1938, found 422.1936.

(S)-Methyl 2-amino-3-(4-((4-methylbenzyl)oxy)phenyl)propanoate (6c). Pale yellow viscous oil, 1.47 g (98%). ¹H NMR (400 MHz, CDCl₃): δ 7.29 (2H, d, *J* = 7.9 Hz, Ar-H), 7.17 (2H, d, *J* = 7.9 Hz, Ar-H), 7.07 (2H, d, *J* = 8.5 Hz, Ar-H), 6.89 (2H, d, *J* = 8.5 Hz, Ar-H), 4.97 (2H, s, CH₂O), 3.74 (1H, dd, *J* = 7.3, 5.3 Hz, CH₂CHNH), 3.70 (3H, s, CH₃O), 3.03 (1H, dd, *J* = 13.8, 5.2 Hz, CH_AH_BCHNH), 2.84 (1H, dd, *J* = 13.8, 7.7 Hz, CH_AH_BCHNH), 2.68 (2H, bs, NH₂), 2.34 (3H, s, CH₃Ph). ¹³C NMR (100 MHz, CDCl₃): δ 174.5 (CO), 158.0 (Ar-C), 137.7 (Ar-C), 133.9 (Ar-C), 130.3 (Ar-C), 129.3 (Ar-C), 128.6 (Ar-C), 127.6 (Ar-C), 115.0 (Ar-C), 69.9 (CH₂O), 55.6 (CH₂CHNH), 52.2 (CH₃O), 39.4 (CH₂CHNH), 21.2 (CH₃Ph). HRMS calculated for C₁₈H₂₂NO₃ [M + H]⁺ 300.1594, found 300.1591.

(S)-Methyl 2-(2-(acetylthio)acetamido)-3-(4-((4-methylbenzyl)oxy)phenyl)propanoate (8c). Off white solid, 1.36 g (82%). ¹H NMR (500 MHz, CDCl₃): δ 7.28 (2H, d, *J* = 7.9 Hz, Ar-H), 7.17 (2H, d, *J* = 7.9 Hz, Ar-H), 6.97 (2H, d, *J* = 8.6 Hz, Ar-H), 6.86 (2H, d, *J* = 8.6 Hz, Ar-H), 6.57 (1H, d, *J* = 7.7 Hz, NH), 4.97 (2H, s, CH₂O), 4.75 (1H, dt, *J* = 7.8, 5.5 Hz, CH₂CHNH), 3.70 (3H, s, CH₃O), 3.51 (2H, dd, *J* = 31.0, 14.8 Hz, CH₂S), 3.06 (1H, dd, *J* = 13.8, 5.5 Hz, CH_AH_BCHNH), 2.99 (1H, dd, *J* = 13.8, 6.1 Hz, CH_AH_BCHNH), 2.34 (3H, s, CH₃CO), 2.31 (3H, s, CH₃Ph). ¹³C NMR (125 MHz, CDCl₃): δ 195.2 (COS), 171.6 (CO₂), 167.6 (CONH), 158.0 (Ar-C), 137.8 (Ar-C), 133.9 (Ar-C), 130.3 (Ar-C), 129.3 (Ar-C), 127.7 (Ar-C), 127.6 (Ar-C), 114.9 (Ar-C), 69.9 (CH₂O), 53.5 (CH₂CHNH), 52.3 (CH₃O), 36.8 (CH₂CHNH), 32.8 (CH₂S), 30.1 (CH₃CO), 21.2 (CH₃Ph). HRMS calculated for C₂₂H₂₅NNaO₅S [M + Na]⁺ 438.1346, found 438.1342.

(S)-2-(2-Mercaptoacetamido)-3-(4-((4-methylbenzyl)oxy)phenyl)propanoic acid (2c). Off white solid, 0.73 g (68%). ¹H NMR (500 MHz, CD₃OD): δ 7.26 (2H, d, *J* = 9.0 Hz, Ar-H), 7.19–7.06 (4H, m, Ar-H), 6.88 (2H, d, *J* = 8.5 Hz, Ar-H), 4.96 (2H, s, ArCH₂O), 4.61 (1H, dd, *J* = 8.3, 5.3 Hz, CH₂CHNH), 3.13 (1H, dd, *J* = 14.0, 6.0), 3.11 (2H, s, CH₂SH), 2.93 (1H, dd, *J* = 14.0, 8.2 Hz, CH_AH_BCHNH), 2.31 (3H, s, CH₃). ¹³C NMR (125 MHz, CD₃OD): δ 174.4 (CO₂H), 172.9 (CONH), 159.2 (Ar-C), 138.7 (Ar-C), 135.7 (Ar-C), 131.4 (Ar-C), 130.2 (Ar-C), 130.1 (Ar-C), 128.7 (Ar-C), 115.9 (Ar-C), 70.9 (PhCH₂O), 55.3 (CH₂CHNH), 37.5 (CH₂CHNH), 28.0 (CH₂SH), 21.2 (CH₃), 1.90 (1H, s, SH). HRMS calculated for C₁₉H₂₀NO₄S [M-H]⁻ 358.1191, found 358.1192.

(S)-Methyl 2-((tert-butoxycarbonyl)amino)-3-(4-((2-chlorobenzyl)oxy)phenyl)propanoate (5d). Off white solid, 2.34 g (82%). ¹H NMR (500 MHz, CDCl₃): δ 7.53 (1H, dd, *J* = 7.1, 1.4 Hz, Ar-H), 7.38 (1H, dd, *J* = 7.1, 1.4 Hz, Ar-H), 7.30–7.20 (2H, m, Ar-H), 7.03 (2H, d, *J* = 8.5 Hz, Ar-H), 6.88 (2H, d, *J* = 8.5 Hz, Ar-H), 5.12 (2H, s, CH₂O), 4.96 (1H, d, *J* = 6.7 Hz, NH), 4.53 (1H, q, *J* = 7.0 Hz, CH₂CHNH), 3.69 (3H, s, CH₃O), 3.04 (1H, dd, *J* = 13.8, 5.8 Hz, CH_AH_BCHNH), 2.97 (1H, dd, *J* = 13.8, 6.0 Hz, CH_AH_BCHNH), 1.40 (9H, s, C(CH₃)₃). ¹³C NMR (125 MHz, CDCl₃): δ 172.4 (CO₂CH₃), 157.6 (NHCO₂), 155.1 (Ar-C), 134.7 (Ar-C), 132.5 (Ar-C), 130.4 (Ar-C), 129.3 (Ar-C), 128.9 (Ar-C), 128.7 (Ar-C), 128.5 (Ar-C), 126.9 (Ar-C), 114.9 (Ar-C), 79.9 (C(CH₃)₃), 67.1 (CH₂O), 54.5 (CH₂CHNH), 52.2 (CH₃O), 37.5 (CH₂CHNH), 28.3 (C(CH₃)₃). HRMS calculated for C₂₂H₂₆ClNNaO₅ [M + Na]⁺ 442.1392, found 442.1397.

(S)-Methyl 2-amino-3-(4-((2-chlorobenzyl)oxy)phenyl)propanoate (6d). Green viscous oil, 1.57 g (98%). ¹H NMR (500 MHz, CDCl₃): δ 7.53 (1H, dd, *J* = 7.1, 1.4 Hz, Ar-H), 7.37 (1H, dd, *J* = 7.1, 1.4 Hz, Ar-H), 7.28–7.20 (2H, m, Ar-H), 7.10 (2H, d, *J* = 8.5 Hz, Ar-H), 6.90 (2H, d, *J* = 8.5 Hz, Ar-H), 5.12 (2H, s, CH₂O), 3.69 (4H, s: 1H for CH₂CHNH and 3H for CH₃O), 3.01 (1H, dd, *J* = 13.6, 5.1 Hz, CH_AH_BCHNH), 2.81 (1H, dd, *J* = 13.7, 7.7 Hz, CH_AH_BCHNH), 1.67 (2H, bs, NH₂). ¹³C NMR (125 MHz, CDCl₃): δ 175.3 (CO), 157.4 (Ar-C), 134.7 (Ar-C), 132.5 (Ar-C), 130.3 (Ar-C), 129.6 (Ar-C), 129.3 (Ar-C), 128.9 (Ar-C), 128.7 (Ar-C), 126.9 (Ar-C), 114.9 (Ar-C), 67.1 (CH₂O), 55.8 (CH₂CHNH), 51.9 (CH₃O), 40.0 (CH₂CHNH). HRMS calculated for C₁₇H₁₉ClNO₃ [M + H]⁺ 320.1048, found 320.1049.

(S)-Methyl 2-(2-(acetylthio)acetamido)-3-(4-((2-chlorobenzyl)oxy)phenyl)propanoate (8d). Yellow viscous oil, 1.43 g (82%). ¹H NMR (500 MHz, CDCl₃): δ 7.53 (1H, dd, *J* = 7.1, 1.4 Hz, Ar-H), 7.37 (1H, dd, *J* = 7.1, 1.4 Hz, Ar-H), 7.28–7.20 (2H, m, Ar-H), 7.00 (2H, d, *J* = 8.5 Hz, Ar-H), 6.88 (2H, d, *J* = 8.5 Hz, Ar-H), 6.60 (1H, d, *J* = 7.7 Hz, NH), 5.12 (2H, s, CH₂O), 4.75 (1H, q, *J* = 6.4 Hz, CH₂CHNH), 3.70 (3H, s, CH₃O), 3.51 (2H, dd, *J* = 32.0, 15.0 Hz, CH₂S), 3.07 (1H, dd, *J* = 13.8, 5.5 Hz, CH_AH_BCHNH), 2.99 (1H, dd, *J* = 13.8, 6.1 Hz, CH_AH_BCHNH), 2.32 (3H, s, CH₃CO). ¹³C NMR (125 MHz, CDCl₃): δ 195.1 (COS), 171.6 (CO₂), 167.6 (CONH), 157.6 (Ar-C), 134.6 (Ar-C), 132.5 (Ar-C), 130.3 (Ar-C), 129.3 (Ar-C), 129.0 (Ar-C), 128.7 (Ar-C), 128.1 (Ar-C), 126.9 (Ar-C), 114.8 (Ar-C), 67.1 (CH₂O), 53.5 (CH₂CHNH), 52.3 (CH₃O), 36.7 (CH₂CHNH), 32.7 (CH₂S), 30.1 (CH₃CO). HRMS calculated for C₂₁H₂₂ClNNaO₅S [M + Na]⁺ 458.0799, found 458.0803.

(S)-3-(4-((2-Chlorobenzyl)oxy)phenyl)-2-(2-mercaptoacetamido)propanoic acid (2d). Tan solid, 0.79 g (69%). ¹H NMR (500 MHz, CD₃OD): δ 7.52 (1H, m, Ar-H), 7.40 (1H, m, Ar-H), 7.31–7.25 (2H, m, Ar-H), 7.14 (2H, d, *J* = 8.5 Hz, Ar-H), 6.90 (2H, d, *J* = 8.5 Hz, Ar-H), 5.10 (2H, s, CH₂O), 4.62 (1H, dd, *J* = 8.0, 5.4 Hz, CH₂CHNH), 3.14

(1H, dd, $J = 13.8$, 5.5 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 3.12 (2H, s, CH_2SH), 2.95 (1H, dd, $J = 13.8$, 6.1 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$). ^{13}C NMR (125 MHz, CD_3OD): δ 174.3 (CO_2H), 172.9 (CONH), 159.0 (Ar-C), 136.1 (Ar-C), 134.0 (Ar-C), 131.5 (Ar-C), 130.6 (Ar-C), 130.4 (Ar-C), 130.4 (Ar-C), 130.3 (Ar-C), 128.1 (Ar-C), 115.9 (Ar-C), 68.3 (PhCH_2O), 55.3 (CH_2CHNH), 37.5 (CH_2CHNH), 28.0 (CH_2SH). HRMS calculated for $\text{C}_{18}\text{H}_{17}\text{ClNO}_4\text{S}$ $[\text{M}-\text{H}]^-$ 378.0572, found 378.0566.

(S)-Methyl 2-((tert-butoxycarbonyl)amino)-3-(4-((3-chlorobenzyl)oxy)phenyl)propanoate (5e). Pale yellow oil, 2.28 g (80%). ^1H NMR (500 MHz, CDCl_3): δ 7.41 (1H, s, Ar-H), 7.28 (3H, s, Ar-H), 7.02 (2H, d, $J = 8.5$ Hz, Ar-H), 6.86 (2H, d, $J = 8.5$ Hz, Ar-H), 4.99 (2H, s, CH_2O), 4.96 (1H, d, $J = 7.5$ Hz, NH), 4.53 (1H, q, $J = 7.2$ Hz, CH_2CHNH), 3.69 (3H, s, CH_3O), 3.03 (1H, dd, $J = 13.8$, 5.8 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 2.98 (1H, dd, $J = 13.8$, 6.0 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 1.40 (9H, s, $\text{C}(\text{CH}_3)_3$). ^{13}C NMR (125 MHz, CDCl_3): δ 172.4 (CO_2CH_3), 157.5 (NHCO_2), 155.1 (Ar-C), 139.1 (Ar-C), 134.5 (Ar-C), 130.3 (Ar-C), 129.8 (Ar-C), 128.6 (Ar-C), 128.0 (Ar-C), 127.4 (Ar-C), 125.3 (Ar-C), 114.9 (Ar-C), 79.9 ($\text{C}(\text{CH}_3)_3$), 69.1 (CH_2O), 54.5 (CH_2CHNH), 52.2 (CH_3O), 37.5 (CH_2CHNH), 28.3 ($\text{C}(\text{CH}_3)_3$). HRMS calculated for $\text{C}_{22}\text{H}_{26}\text{ClNNaO}_5$ $[\text{M} + \text{Na}]^+$ 442.1392, found 442.1394.

(S)-Methyl 2-amino-3-(4-((3-chlorobenzyl)oxy)phenyl)propanoate (6e). Yellow solid, 1.57 g (98%). ^1H NMR (500 MHz, CDCl_3): δ 7.40 (1H, s, Ar-H), 7.26 (3H, s, Ar-H), 7.08 (2H, d, $J = 8.6$ Hz, Ar-H), 6.87 (2H, d, $J = 8.6$ Hz, Ar-H), 4.97 (2H, s, CH_2O), 3.68 (3H, s, CH_3O), 3.66 (1H, m, CH_2CHNH), 3.00 (1H, dd, $J = 13.6$, 5.1 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 2.80 (1H, dd, $J = 13.6$, 7.7 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 1.65 (2H, bs, NH_2). ^{13}C NMR (125 MHz, CDCl_3): δ 175.3 (CO), 157.3 (Ar-C), 139.0 (Ar-C), 134.4 (Ar-C), 130.2 (Ar-C), 129.7 (Ar-C), 129.6 (Ar-C), 127.9 (Ar-C), 127.3 (Ar-C), 125.2 (Ar-C), 114.8 (Ar-C), 69.0 (CH_2O), 55.7 (CH_2CHNH), 51.8 (CH_3O), 40.0 (CH_2CHNH). HRMS calculated for $\text{C}_{17}\text{H}_{19}\text{ClNO}_3$ $[\text{M} + \text{H}]^+$ 320.1048, found 320.1047.

(S)-Methyl 2-(2-(acetylthio)acetamido)-3-(4-((3-chlorobenzyl)oxy)phenyl)propanoate (8e). Brown viscous oil, 1.45 g (83%). ^1H NMR (500 MHz, CDCl_3): δ 7.40 (1H, s, Ar-H), 7.29 (3H, s, Ar-H), 6.99 (2H, d, $J = 8.6$ Hz, Ar-H), 6.85 (2H, d, $J = 8.6$ Hz, Ar-H), 6.59 (1H, d, $J = 7.7$ Hz, NH), 4.99 (2H, s, CH_2O), 4.76 (1H, dt, $J = 7.7$, 5.9 Hz, CH_2CHNH), 3.70 (3H, s, CH_3O), 3.51 (2H, dd, $J = 27.5$, 14.8 Hz, CH_2S), 3.07 (1H, dd, $J = 13.8$, 5.5 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 2.99 (1H, dd, $J = 13.8$, 6.1 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 2.32 (3H, s, CH_3CO). ^{13}C NMR (125 MHz, CDCl_3): δ 195.2 (COS), 171.5 (CO_2), 167.6 (CONH), 157.6 (Ar-C), 139.0 (Ar-C), 134.5 (Ar-C), 130.4 (Ar-C), 129.9 (Ar-C), 128.2 (Ar-C), 128.1 (Ar-C), 127.4 (Ar-C), 125.3 (Ar-C), 114.8 (Ar-C), 69.1 (CH_2O), 53.5 (CH_2CHNH), 52.4 (CH_3O), 36.8 (CH_2CHNH), 32.8 (CH_2S), 30.1 (CH_3CO). HRMS calculated for $\text{C}_{21}\text{H}_{22}\text{ClNNaO}_5\text{S}$ $[\text{M} + \text{Na}]^+$ 458.0799, found 458.0800.

(S)-3-(4-((3-chlorobenzyl)oxy)phenyl)-2-(2-mercaptoacetamido)propanoic acid (2e). Tan solid, 0.77 g (68%). ^1H NMR (500 MHz, CD_3OD): δ 7.42 (1H, s, Ar-H), 7.33–7.26 (3H, m, Ar-H), 7.13 (2H, d, $J = 8.6$ Hz, Ar-H), 6.89 (2H, d, $J = 8.6$ Hz, Ar-H), 5.02 (2H, s, ArCH_2O), 4.62 (1H, dd, $J = 8.2$, 5.2 Hz, CH_2CHNH), 3.14 (1H, dd, $J = 18.0$, 6.0 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 3.12 (2H, s, CH_2SH), 2.94 (1H, dd, $J = 18.0$, 8.0 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$). ^{13}C NMR (125 MHz, CD_3OD): δ 174.3 (CO_2H), 172.9 (CONH), 158.9 (Ar-C), 141.2 (Ar-C), 135.4 (Ar-C), 131.5 (Ar-C), 131.0 (Ar-C), 130.6 (Ar-C), 128.8 (Ar-C), 128.3 (Ar-C), 126.6 (Ar-C), 115.9 (Ar-C), 70.0 (PhCH_2O), 55.3 (CH_2CHNH), 37.5 (CH_2CHNH), 28.0 (CH_2SH). HRMS calculated for $\text{C}_{18}\text{H}_{17}\text{ClNO}_4\text{S}$ $[\text{M}-\text{H}]^-$ 378.0572, found 378.0569.

(S)-Methyl 2-((tert-butoxycarbonyl)amino)-3-(4-((4-chlorobenzyl)oxy)phenyl)propanoate (5f). Yellow solid, 2.28 g (82%). ^1H NMR (400 MHz, CDCl_3): δ 7.33 (4H, s, Ar-H), 7.02 (2H, d, $J = 8.5$ Hz, Ar-H), 6.85 (2H, d, $J = 8.5$ Hz, Ar-H), 4.98 (2H, s, CH_2O), 4.94 (1H, d, $J = 7.5$ Hz, NH), 4.59–4.46 (1H, m, CH_2CHNH), 3.69 (3H, s, CH_3O), 3.05 (1H, dd, $J = 13.8$, 5.8 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 2.95 (1H, dd, $J = 13.8$, 6.0 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 1.40 (9H, s, $\text{C}(\text{CH}_3)_3$). ^{13}C NMR (100 MHz, CDCl_3): δ 172.4 (CO_2CH_3), 157.6 (NHCO_2), 155.1 (Ar-C), 135.5 (Ar-C), 133.7 (Ar-C), 130.3 (Ar-C), 128.7 (Ar-C), 128.5 (Ar-C), 128.2 (Ar-C), 114.9 (Ar-C), 79.9 ($\text{C}(\text{CH}_3)_3$), 69.2 (CH_2O), 54.5 (CH_2CHNH), 52.2 (CH_3O), 37.5 (CH_2CHNH), 28.3 ($\text{C}(\text{CH}_3)_3$). HRMS calculated for $\text{C}_{22}\text{H}_{26}\text{ClNNaO}_5$ $[\text{M} + \text{Na}]^+$ 442.1392, found 442.1395.

(S)-Methyl 2-amino-3-(4-((4-chlorobenzyl)oxy)phenyl)propanoate (6f). Brown solid, 1.57 g (98%). ^1H NMR (500 MHz, CDCl_3): δ 7.33 (4H, s, Ar-H), 7.08 (2H, d, $J = 8.6$ Hz, Ar-H), 6.87 (2H, d, $J = 8.6$ Hz, Ar-H), 4.98 (2H, s, CH_2O), 3.69 (4H, s, (1H for CH_2CHNH and 3H for CH_3O)), 3.01 (1H, dd, $J = 13.8$, 5.8 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 2.80 (1H, dd, $J = 13.8$, 6.0 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 1.71 (2H, s, NH_2). ^{13}C NMR (125 MHz, CDCl_3): δ 175.3 (CO), 157.5 (Ar-C), 135.5 (Ar-C), 133.7 (Ar-C), 130.3 (Ar-C), 129.6 (Ar-C), 128.9 (Ar-C), 128.7 (Ar-C), 114.9 (Ar-C), 69.2 (CH_2O), 55.8 (CH_2CHNH), 52.0 (CH_3O), 40.0 (CH_2CHNH). HRMS calculated for $\text{C}_{17}\text{H}_{19}\text{ClNO}_3$ $[\text{M} + \text{H}]^+$ 320.1048, found 320.1046.

(S)-Methyl 2-(2-(acetylthio)acetamido)-3-(4-((4-chlorobenzyl)oxy)phenyl)propanoate (8f). Brown viscous oil, 1.39 g (80%). ^1H NMR (500 MHz, CDCl_3): δ 7.33 (4H, s, Ar-H), 6.98 (2H, d, $J = 8.7$ Hz, Ar-H), 6.84 (2H, d, $J = 8.7$ Hz, Ar-H), 6.59 (1H, d, $J = 7.7$ Hz, NH), 4.98 (2H, s, CH_2O), 4.75 (1H, dt, $J = 7.8$, 5.5 Hz, CH_2CHNH), 3.70 (3H, s, CH_3O), 3.51 (2H, dd, $J = 27.5$, 14.2 Hz, CH_2S), 3.07 (1H, dd, $J = 13.8$, 5.5 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 2.98 (1H, dd, $J = 13.8$, 6.1 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 2.32 (3H, s, CH_3CO). ^{13}C NMR (125 MHz, CDCl_3): δ 195.2 (COS), 171.6 (CO_2), 167.7 (CONH), 157.7 (Ar-C), 135.4 (Ar-C), 133.8 (Ar-C), 130.4 (Ar-C), 130.3 (Ar-C), 128.7 (Ar-C), 128.1 (Ar-C), 114.9 (Ar-C), 69.2 (CH_2O), 53.5 (CH_2CHNH), 52.4 (CH_3O), 36.8 (CH_2CHNH), 32.7 (CH_2S), 30.1 (CH_3CO). HRMS calculated for $\text{C}_{21}\text{H}_{22}\text{ClNNaO}_5\text{S}$ $[\text{M} + \text{Na}]^+$ 458.0799, found 458.0806.

(S)-3-(4-((4-Chlorobenzyl)oxy)phenyl)-2-(2-mercaptoacetamido)propanoic acid (2f). Off white solid, 0.80 g (70%). ^1H NMR (500 MHz, CD_3OD): δ 7.39 (2H, d, $J = 8.5$ Hz, Ar-H), 7.34 (2H, d, $J = 8.5$ Hz, Ar-H), 7.13 (2H, d, $J = 8.5$ Hz, Ar-H), 6.89 (2H, d, $J = 8.5$ Hz, Ar-H), 5.02 (2H, s, ArCH_2O), 4.61 (1H, dd, $J = 8.3$, 5.3 Hz, CH_2CHNH), 3.14 (1H, $J = 14.0$, 5.5 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 3.13 (2H, s, CH_2SH), 2.94 (1H, dd, $J = 14.0$, 8.2 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$). ^{13}C NMR (125 MHz, CD_3OD): δ 174.3 (CO_2H), 172.9 (CONH), 159.0 (Ar-C), 137.6 (Ar-C), 134.6 (Ar-C), 131.5 (Ar-C), 130.5 (Ar-C), 130.1 (Ar-C), 129.6 (Ar-C), 115.9 (Ar-C), 70.1 (PhCH_2O), 55.3 (CH_2CHNH), 37.5 (CH_2CHNH), 28.00 (CH_2SH). HRMS calculated for $\text{C}_{18}\text{H}_{17}\text{ClNO}_4\text{S}$ $[\text{M}-\text{H}]^-$ 378.0572, found 378.0557.

(S)-Methyl 2-((tert-butoxycarbonyl)amino)-3-(4-((2-nitrobenzyl)oxy)phenyl)propanoate (5g). Pale yellow viscous oil, 2.78 g (95%). ^1H NMR (500 MHz, CDCl_3): δ 8.13 (1H, d, $J = 8.0$ Hz, Ar-H), 7.85 (1H, d, $J = 7.8$ Hz, Ar-H), 7.65 (1H, t, $J = 7.6$ Hz, Ar-H), 7.44 (1H, t, $J = 8.0$ Hz, Ar-H), 7.03 (2H, d, $J = 8.5$ Hz, Ar-H), 6.88 (2H, d, $J = 8.5$ Hz, Ar-H), 5.44 (2H, s, CH_2O), 4.97 (1H, d, $J = 6.7$ Hz, NH), 4.52 (1H, q, $J = 7.0$ Hz, CH_2CHNH), 3.69 (3H, s, CH_3O), 3.04 (1H, dd, $J = 13.8$, 5.8 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 2.97 (1H, dd, $J = 13.8$, 6.0 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 1.39 (9H, s, $\text{C}(\text{CH}_3)_3$). ^{13}C NMR (125 MHz, CDCl_3): δ 172.3 (CO_2CH_3), 157.2 (NHCO_2), 155.0 (Ar-C), 146.8 (Ar-C), 134.0 (Ar-C), 133.9 (Ar-C), 130.4 (Ar-C), 128.9 (Ar-C), 128.5 (Ar-C), 128.3 (Ar-C), 124.9 (Ar-C), 114.9 (Ar-C), 79.9 ($\text{C}(\text{CH}_3)_3$), 66.8 (CH_2O), 54.5 (CH_2CHNH), 52.2

(CH₃O), 37.4 (CH₂CHNH), 28.3 (C(CH₃)₃). HRMS calculated for C₂₂H₂₆N₂NaO₇ [M + Na]⁺ 453.1632, found 453.1635.

(S)-Methyl 2-amino-3-(4-((2-nitrobenzyl)oxy)phenyl)propanoate (6g). Tan yellow viscous oil, 1.62 g (98%). ¹H NMR (500 MHz, CDCl₃): δ 8.13 (1H, d, *J* = 8.0 Hz, Ar-H), 7.86 (1H, d, *J* = 7.8 Hz, Ar-H), 7.65 (1H, t, *J* = 7.6 Hz, Ar-H), 7.46 (1H, t, *J* = 8.0 Hz, Ar-H), 7.10 (2H, d, *J* = 8.5 Hz, Ar-H), 6.90 (2H, d, *J* = 8.5 Hz, Ar-H), 5.44 (2H, s, CH₂O), 3.69 (4H, s: 1H for CH₂CHNH and 3H for CH₃O), 3.01 (1H, dd, *J* = 13.6, 5.1 Hz, CH_AH_BCHNH), 2.82 (1H, dd, *J* = 13.7, 7.7 Hz, CH_AH_BCHNH), 1.95 (2H, bs, NH₂). ¹³C NMR (125 MHz, CDCl₃): δ 175.2 (CO), 157.1 (Ar-C), 146.9 (Ar-C), 134.0 (Ar-C), 133.9 (Ar-C), 130.4 (Ar-C), 129.9 (Ar-C), 128.5 (Ar-C), 128.3 (Ar-C), 124.9 (Ar-C), 115.0 (Ar-C), 66.8 (CH₂O), 55.7 (CH₂CHNH), 52.0 (CH₃O), 39.9 (CH₂CHNH). HRMS calculated for C₁₇H₁₉N₂O₅ [M + H]⁺ 331.1288, found 331.1290.

(S)-Methyl 2-(2-(acetylthio)acetamido)-3-(4-((2-nitrobenzyl)oxy)phenyl)propanoate (8g). Pale yellow solid, 1.48 g (83%). ¹H NMR (500 MHz, CDCl₃): δ 8.13 (1H, d, *J* = 8.0 Hz, Ar-H), 7.86 (1H, d, *J* = 7.8 Hz, Ar-H), 7.66 (1H, t, *J* = 7.6 Hz, Ar-H), 7.47 (1H, t, *J* = 8.0 Hz, Ar-H), 7.00 (2H, d, *J* = 8.5 Hz, Ar-H), 6.88 (2H, d, *J* = 8.5 Hz, Ar-H), 6.59 (1H, d, *J* = 7.6 Hz, NH), 5.43 (2H, s, CH₂O), 4.76 (1H, dt, *J* = 7.8, 5.8 Hz, CH₂CHNH), 3.70 (3H, s, CH₃O), 3.51 (2H, dd, *J* = 28.2, 15.0 Hz, CH₂S), 3.08 (1H, dd, *J* = 13.8, 5.5 Hz, CH_AH_BCHNH), 2.99 (1H, dd, *J* = 13.8, 6.1 Hz, CH_AH_BCHNH), 2.33 (3H, s, CH₃CO). ¹³C NMR (125 MHz, CDCl₃): δ 195.2 (COS), 171.5 (CO₂), 167.6 (CONH), 157.3 (Ar-C), 146.9 (Ar-C), 134.0 (Ar-C), 133.8 (Ar-C), 130.5 (Ar-C), 128.5 (Ar-C), 128.5 (Ar-C), 128.3 (Ar-C), 125.0 (Ar-C), 114.9 (Ar-C), 66.8 (CH₂O), 53.5 (CH₂CHNH), 52.4 (CH₃O), 36.8 (CH₂CHNH), 32.8 (CH₂S), 30.2 (CH₃CO). HRMS calculated for C₂₁H₂₂N₂NaO₇S [M + Na]⁺ 469.1040, found 469.1045.

(S)-2-(2-Mercaptoacetamido)-3-(4-((2-nitrobenzyl)oxy)phenyl)propanoic acid (2g). Pale yellow solid, 0.79 g (67%). ¹H NMR (500 MHz, CDCl₃): δ 8.22 (1H, bs, OH), 8.12 (1H, d, *J* = 8.0 Hz, Ar-H), 7.84 (1H, d, *J* = 7.8 Hz, Ar-H), 7.64 (1H, t, *J* = 7.6 Hz, Ar-H), 7.45 (1H, t, *J* = 8.0 Hz, Ar-H), 7.13 (1H, d, *J* = 7.6 Hz, NH), 7.00 (2H, d, *J* = 8.5 Hz, Ar-H), 6.89 (2H, d, *J* = 8.5 Hz, Ar-H), 5.41 (2H, s, CH₂O), 4.80 (1H, q, *J* = 6.4 Hz, CH₂CHNH), 3.20 (2H, d, *J* = 9.1 Hz, CH₂SH), 3.16 (1H, dd, *J* = 13.8, 5.5 Hz, CH_AH_BCHNH), 3.07 (1H, dd, *J* = 13.8, 6.1 Hz, CH_AH_BCHNH), 1.83 (3H, t, *J* = 9.1 Hz, SH). ¹³C NMR (125 MHz, CDCl₃): δ 177.0 (CO₂H), 170.0 (CONH), 157.4 (Ar-C), 146.9 (Ar-C), 134.0 (Ar-C), 133.7 (Ar-C), 130.6 (Ar-C), 128.5 (Ar-C), 128.3 (Ar-C), 128.3 (Ar-C), 124.9 (Ar-C), 115.1 (Ar-C), 66.8 (PhCH₂O), 53.5 (CH₂CHNH), 36.4 (CH₂CHNH), 28.1 (CH₂SH). HRMS calculated for C₁₈H₁₇N₂O₆S [M-H]⁻ 389.0813, found 389.0809.

(S)-Methyl 2-((tert-butoxycarbonyl)amino)-3-(4-((3-nitrobenzyl)oxy)phenyl)propanoate (5h). Yellow viscous oil, 2.63 g (90%). ¹H NMR (500 MHz, CDCl₃): δ 8.27 (1H, s, Ar-H), 8.15 (1H, d, *J* = 8.2 Hz, Ar-H), 7.72 (1H, d, *J* = 7.6 Hz, Ar-H), 7.52 (1H, t, *J* = 7.9 Hz, Ar-H), 7.03 (2H, d, *J* = 8.5 Hz, Ar-H), 6.86 (2H, d, *J* = 8.5 Hz, Ar-H), 5.09 (2H, s, CH₂O), 4.98 (1H, d, *J* = 7.9 Hz, NH), 4.51 (1H, q, *J* = 7.0 Hz, CH₂CHNH), 3.67 (3H, s, CH₃O), 3.03 (1H, dd, *J* = 13.8, 5.8 Hz, CH_AH_BCHNH), 2.96 (1H, dd, *J* = 13.8, 6.0 Hz, CH_AH_BCHNH), 1.38 (9H, s, C(CH₃)₃). ¹³C NMR (125 MHz, CDCl₃): δ 172.3 (CO₂CH₃), 157.2 (NHCO₂), 155.0 (Ar-C), 148.3 (Ar-C), 139.2 (Ar-C), 133.0 (Ar-C), 130.4 (Ar-C), 129.5 (Ar-C), 128.9 (Ar-C), 122.8 (Ar-C), 122.0 (Ar-C), 114.8 (Ar-C), 79.8 (C(CH₃)₃), 68.5 (CH₂O), 54.4 (CH₂CHNH), 52.1 (CH₃O), 37.4 (CH₂CHNH), 28.2 (C(CH₃)₃). HRMS calculated for C₂₂H₂₆N₂NaO₇ [M + Na]⁺ 453.1632, found 453.1639.

(S)-Methyl 2-amino-3-(4-((3-nitrobenzyl)oxy)phenyl)propanoate (6h). Brown solid, 1.62 g (98%). ¹H NMR (400 MHz, CDCl₃): δ 8.26 (1H, s, Ar-H), 8.12 (1H, d, *J* = 8.2 Hz, Ar-H), 7.72 (1H, d, *J* = 7.6 Hz, Ar-H), 7.52 (1H, t, *J* = 7.9 Hz, Ar-H), 7.09 (2H, d, *J* = 8.5 Hz, Ar-H), 6.87 (2H, d, *J* = 8.5 Hz, Ar-H), 5.08 (2H, s, CH₂O), 3.72 (1H, dd, *J* = 7.4, 5.1 Hz, CH₂CHNH), 3.67 (3H, s, CH₃O), 3.02 (1H, dd, *J* = 13.6, 5.1 Hz, CH_AH_BCHNH), 2.84 (1H, dd, *J* = 13.7, 7.7 Hz, CH_AH_BCHNH), 2.75 (2H, bs, NH₂). ¹³C NMR (100 MHz, CDCl₃): δ 174.5 (CO), 157.1 (Ar-C), 148.3 (Ar-C), 139.1 (Ar-C), 133.0 (Ar-C), 130.4 (Ar-C), 129.5 (Ar-C), 122.8 (Ar-C), 122.0 (Ar-C), 121.2 (Ar-C), 114.8 (Ar-C), 68.5 (CH₂O), 55.4 (CH₂CHNH), 52.0 (CH₃O), 39.3 (CH₂CHNH). HRMS calculated for C₁₇H₁₉N₂O₅ [M + H]⁺ 331.1288, found 331.1286.

(S)-Methyl 2-(2-(acetylthio)acetamido)-3-(4-((3-nitrobenzyl)oxy)phenyl)propanoate (8h). Yellow solid, 1.45 g (81%). ¹H NMR (400 MHz, CDCl₃): δ 8.28 (1H, s, Ar-H), 8.15 (1H, d, *J* = 8.2 Hz, Ar-H), 7.73 (1H, d, *J* = 7.6 Hz, Ar-H), 7.54 (1H, t, *J* = 7.9 Hz, Ar-H), 7.00 (2H, d, *J* = 8.5 Hz, Ar-H), 6.88 (2H, d, *J* = 8.5 Hz, Ar-H), 6.62 (1H, d, *J* = 7.6 Hz, NH), 5.10 (2H, s, CH₂O), 4.74 (1H, dt, *J* = 7.8, 5.8 Hz, CH₂CHNH), 3.69 (3H, s, CH₃O), 3.51 (2H, dd, *J* = 22.8, 14.9 Hz, CH₂S), 3.07 (1H, dd, *J* = 13.8, 5.5 Hz, CH_AH_BCHNH), 2.98 (1H, dd, *J* = 13.8, 6.1 Hz, CH_AH_BCHNH), 2.33 (3H, s, CH₃CO). ¹³C NMR (100 MHz, CDCl₃): δ 195.2 (COS), 171.5 (CO₂), 167.6 (CONH), 157.3 (Ar-C), 148.4 (Ar-C), 139.1 (Ar-C), 133.1 (Ar-C), 130.5 (Ar-C), 129.5 (Ar-C), 128.6 (Ar-C), 122.9 (Ar-C), 122.1 (Ar-C), 114.8 (Ar-C), 68.6 (CH₂O), 53.5 (CH₂CHNH), 52.4 (CH₃O), 36.8 (CH₂CHNH), 32.7 (CH₂S), 30.1 (CH₃CO). HRMS calculated for C₂₁H₂₂N₂NaO₇S [M + Na]⁺ 469.1040, found 469.1045.

(S)-2-(2-Mercaptoacetamido)-3-(4-((3-nitrobenzyl)oxy)phenyl)propanoic acid (2h). Pale yellow solid, 0.82 g (70%). ¹H NMR (400 MHz, CDCl₃): δ 10.56 (1H, bs, OH), 8.25 (1H, s, Ar-H), 8.13 (1H, d, *J* = 8.2 Hz, Ar-H), 7.71 (1H, d, *J* = 7.6 Hz, Ar-H), 7.52 (1H, t, *J* = 7.9 Hz, Ar-H), 7.20 (1H, d, *J* = 7.8 Hz, NH), 7.09 (2H, d, *J* = 8.5 Hz, Ar-H), 6.88 (2H, d, *J* = 8.5 Hz, Ar-H), 5.07 (2H, s, CH₂O), 4.80 (1H, dt, *J* = 7.8, 5.2 Hz, CH₂CHNH), 3.20 (2H, d, *J* = 9.1 Hz, CH₂SH), 3.16 (1H, dd, *J* = 13.8, 5.5 Hz, CH_AH_BCHNH), 3.07 (1H, dd, *J* = 13.8, 6.1 Hz, CH_AH_BCHNH), 1.84 (3H, t, *J* = 9.1 Hz, SH). ¹³C NMR (100 MHz, CDCl₃): δ 174.8 (CO₂H), 170.3 (CONH), 157.4 (Ar-C), 148.3 (Ar-C), 139.0 (Ar-C), 133.1 (Ar-C), 130.5 (Ar-C), 129.5 (Ar-C), 128.2 (Ar-C), 122.9 (Ar-C), 122.1 (Ar-C), 114.9 (Ar-C), 68.5 (PhCH₂O), 53.5 (CH₂CHNH), 36.3 (CH₂CHNH), 28.0 (CH₂SH). HRMS calculated for C₁₈H₁₇N₂O₆S [M-H]⁻ 389.0813, found 389.0805.

(S)-Methyl 2-((tert-butoxycarbonyl)amino)-3-(4-((4-nitrobenzyl)oxy)phenyl)propanoate (5i). Brown viscous oil, 2.69 g (92%). ¹H NMR (500 MHz, CDCl₃): δ 8.21 (2H, d, *J* = 8.7 Hz, Ar-H), 7.56 (2H, d, *J* = 8.7 Hz, Ar-H), 7.03 (2H, d, *J* = 8.5 Hz, Ar-H), 6.86 (2H, d, *J* = 8.5 Hz, Ar-H), 5.12 (2H, s, CH₂O), 4.96 (1H, d, *J* = 7.3 Hz, NH), 4.50 (1H, q, *J* = 7.6 Hz, CH₂CHNH), 3.68 (3H, s, CH₃O), 3.03 (1H, dd, *J* = 13.8, 5.8 Hz, CH_AH_BCHNH), 2.97 (1H, dd, *J* = 13.8, 6.0 Hz, CH_AH_BCHNH), 1.38 (9H, s, C(CH₃)₃). ¹³C NMR (125 MHz, CDCl₃): δ 172.3 (CO₂CH₃), 157.2 (NHCO₂), 155.0 (Ar-C), 147.5 (Ar-C), 144.5 (Ar-C), 130.4 (Ar-C), 129.0 (Ar-C), 127.5 (Ar-C), 123.8 (Ar-C), 114.8 (Ar-C), 79.9 (C(CH₃)₃), 68.6 (CH₂O), 54.5 (CH₂CHNH), 52.2 (CH₃O), 37.4 (CH₂CHNH), 28.2 (C(CH₃)₃). HRMS calculated for C₂₂H₂₆N₂NaO₇ [M + Na]⁺ 453.1632, found 453.1636.

(S)-Methyl 2-amino-3-(4-((4-nitrobenzyl)oxy)phenyl)propanoate (6i). Dark brown solid, 1.62 g (98%). ¹H NMR (400 MHz, CDCl₃): δ 8.16 (2H, d, *J* = 8.7 Hz, Ar-H), 7.80 (2H, bs, NH₂), 7.53 (2H, d, *J* = 8.7 Hz, Ar-H), 7.08 (2H, d, *J* = 8.5 Hz, Ar-H), 6.87 (2H, d,

$J=8.5$ Hz, Ar-H), 5.05 (2H, s, CH_2O), 4.28 (1H, t, $J=6.4$ Hz, CH_2CHNH), 3.77 (3H, s, CH_3O), 3.26 (1H, dd, $J=13.6$, 5.1 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 3.12 (1H, dd, $J=13.7$, 7.7 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$). ^{13}C NMR (100 MHz, CDCl_3): δ 169.5 (CO), 158.1 (Ar-C), 147.4 (Ar-C), 144.3 (Ar-C), 130.4 (Ar-C), 127.5 (Ar-C), 125.1 (Ar-C), 123.8 (Ar-C), 115.5 (Ar-C), 68.5 (CH_2O), 54.7 (CH_2CHNH), 53.6 (CH_3O), 35.2 (CH_2CHNH). HRMS calculated for $\text{C}_{17}\text{H}_{19}\text{N}_2\text{O}_5$ $[\text{M} + \text{H}]^+$ 331.1287, found 331.1287.

(S)-Methyl 2-(2-(acetylthio)acetamido)-3-(4-((4-nitrobenzyl)oxy)phenyl)propanoate (8i). Brown viscous oil, 1.48 g (83%). ^1H NMR (400 MHz, CDCl_3): δ 8.21 (2H, d, $J=8.7$ Hz, Ar-H), 7.57 (2H, d, $J=8.7$ Hz, Ar-H), 7.00 (2H, d, $J=8.5$ Hz, Ar-H), 6.85 (2H, d, $J=8.5$ Hz, Ar-H), 6.60 (1H, d, $J=7.7$ Hz, NH), 5.11 (2H, s, CH_2O), 4.75 (1H, dt, $J=7.8$, 5.2 Hz, CH_2CHNH), 3.69 (3H, s, CH_3O), 3.51 (2H, dd, $J=19.9$, 14.8 Hz, CH_2S), 3.07 (1H, dd, $J=13.8$, 5.5 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 2.97 (1H, dd, $J=13.8$, 6.1 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 2.33 (3H, s, CH_3CO). ^{13}C NMR (100 MHz, CDCl_3): δ 195.1 (COS), 171.5 (CO_2), 167.5 (CONH), 157.2 (Ar-C), 147.5 (Ar-C), 144.4 (Ar-C), 130.4 (Ar-C), 128.6 (Ar-C), 127.5 (Ar-C), 123.8 (Ar-C), 114.8 (Ar-C), 68.6 (CH_2O), 53.5 (CH_2CHNH), 52.3 (CH_3O), 36.8 (CH_2CHNH), 32.7 (CH_2S), 30.1 (CH_3CO). HRMS calculated for $\text{C}_{21}\text{H}_{22}\text{N}_2\text{NaO}_7\text{S}$ $[\text{M} + \text{Na}]^+$ 469.1040, found 469.1042.

(S)-2-(2-Mercaptoacetamido)-3-(4-((4-nitrobenzyl)oxy)phenyl)propanoic acid (2i). Brown solid, 0.84 g (72%). ^1H NMR (400 MHz, CDCl_3): δ 9.43 (1H, bs, OH), 8.21 (2H, d, $J=8.7$ Hz, Ar-H), 7.57 (2H, d, $J=8.7$ Hz, Ar-H), 7.09 (2H, d, $J=8.5$ Hz, Ar-H), 6.88 (2H, d, $J=8.5$ Hz, Ar-H), 5.12 (2H, s, ArCH_2O), 4.81 (1H, dd, $J=7.6$, 6.0 Hz, CH_2CHNH), 3.21 (2H, d, $J=9.1$ Hz, CH_2SH), 3.17 (1H, dd, $J=14.0$, 5.7 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 3.08 (1H, dd, $J=14.0$, 8.0 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 1.81 (1H, t, $J=9.1$ Hz, SH). ^{13}C NMR (100 MHz, CDCl_3): δ 175.4 (CO_2H), 169.9 (CONH), 157.4 (Ar-C), 147.6 (Ar-C), 144.3 (Ar-C), 130.6 (Ar-C), 128.3 (Ar-C), 127.6 (Ar-C), 123.8 (Ar-C), 115.0 (Ar-C), 68.6 (PhCH_2O), 53.5 (CH_2CHNH), 36.4 (CH_2CHNH), 28.1 (CH_2SH). HRMS calculated for $\text{C}_{18}\text{H}_{17}\text{N}_2\text{O}_6\text{S}$ $[\text{M}-\text{H}]^-$ 389.0813, found 389.0809.

(S)-Methyl 3-(4-((3-bromobenzyl)oxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoate (5j). Tan viscous oil, 2.84 g (90%). ^1H NMR (500 MHz, CDCl_3): δ 7.56 (1H, s, Ar-H), 7.43 (1H, d, $J=8.0$ Hz, Ar-H), 7.32 (1H, d, $J=7.7$ Hz, Ar-H), 7.22 (1H, t, $J=8.0$ Hz, Ar-H), 7.02 (2H, d, $J=8.5$ Hz, Ar-H), 6.86 (2H, d, $J=8.5$ Hz, Ar-H), 4.98 (2H, s, CH_2O), 4.94 (1H, d, $J=7.5$ Hz, NH), 4.53 (1H, q, $J=7.2$ Hz, CH_2CHNH), 3.68 (3H, s, CH_3O), 3.03 (1H, dd, $J=13.8$, 5.8 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 2.97 (1H, dd, $J=13.8$, 6.0 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 1.40 (9H, s, $\text{C}(\text{CH}_3)_3$). ^{13}C NMR (125 MHz, CDCl_3): δ 172.4 (CO_2CH_3), 157.5 (NHCO_2), 155.1 (Ar-C), 139.3 (Ar-C), 131.0 (Ar-C), 130.4 (Ar-C), 130.3 (Ar-C), 130.1 (Ar-C), 128.6 (Ar-C), 125.8 (Ar-C), 122.6 (Ar-C), 114.9 (Ar-C), 79.9 ($\text{C}(\text{CH}_3)_3$), 69.1 (CH_2O), 54.5 (CH_2CHNH), 52.2 (CH_3O), 37.5 (CH_2CHNH), 28.3 ($\text{C}(\text{CH}_3)_3$). HRMS calculated for $\text{C}_{22}\text{H}_{26}\text{BrNNaO}_5$ $[\text{M} + \text{Na}]^+$ 486.0887, found 486.0893.

(S)-Methyl 2-amino-3-(4-((3-bromobenzyl)oxy)phenyl)propanoate (6j). Brown solid, 1.78 g (98%). ^1H NMR (500 MHz, CDCl_3): δ 7.56 (1H, s, Ar-H), 7.42 (1H, d, $J=8.0$ Hz, Ar-H), 7.32 (1H, d, $J=7.7$ Hz, Ar-H), 7.22 (1H, t, $J=7.8$ Hz, Ar-H), 7.09 (2H, d, $J=8.6$ Hz, Ar-H), 6.87 (2H, d, $J=8.6$ Hz, Ar-H), 4.98 (2H, s, CH_2O), 3.69 (4H, 3H-singlet for CH_3O and 1H-multiplet for CH_2CHNH), 3.01 (1H, dd, $J=13.6$, 5.1 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 2.82 (1H, dd, $J=13.7$, 7.7 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 1.90 (2H, bs, NH_2). ^{13}C NMR (125 MHz, CDCl_3): δ 175.2 (CO), 157.4 (Ar-C), 139.3 (Ar-C), 130.9 (Ar-C), 130.3 (Ar-C), 130.3 (Ar-C), 130.1 (Ar-C), 129.6 (Ar-C), 125.8 (Ar-C), 122.6 (Ar-C),

114.9 (Ar-C), 69.0 (CH_2O), 55.7 (CH_2CHNH), 52.0 (CH_3O), 39.9 (CH_2CHNH). HRMS calculated for $\text{C}_{17}\text{H}_{19}\text{BrNO}_3$ $[\text{M} + \text{H}]^+$ 364.0543, found 364.0547.

(S)-Methyl 2-(2-(acetylthio)acetamido)-3-(4-((3-bromobenzyl)oxy)phenyl)propanoate (8j). Tan viscous oil, 1.63 g (85%). ^1H NMR (400 MHz, CDCl_3): δ 7.56 (1H, s, Ar-H), 7.42 (1H, d, $J=8.0$ Hz, Ar-H), 7.31 (1H, d, $J=7.7$ Hz, Ar-H), 7.22 (1H, t, $J=7.8$ Hz, Ar-H), 6.98 (2H, d, $J=8.6$ Hz, Ar-H), 6.84 (2H, d, $J=8.6$ Hz, Ar-H), 6.61 (1H, d, $J=7.7$ Hz, NH), 4.97 (2H, s, CH_2O), 4.75 (1H, dt, $J=7.8$, 5.9 Hz, CH_2CHNH), 3.69 (3H, s, CH_3O), 3.51 (2H, dd, $J=24.1$, 14.8 Hz, CH_2S), 3.07 (1H, dd, $J=13.8$, 5.5 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 2.98 (1H, dd, $J=13.8$, 6.1 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 2.32 (3H, s, CH_3CO). ^{13}C NMR (100 MHz, CDCl_3): δ 195.2 (COS), 171.5 (CO_2), 167.6 (CONH), 157.5 (Ar-C), 139.3 (Ar-C), 131.0 (Ar-C), 130.3 (Ar-C), 130.3 (Ar-C), 130.1 (Ar-C), 128.2 (Ar-C), 125.8 (Ar-C), 122.6 (Ar-C), 114.8 (Ar-C), 69.0 (CH_2O), 53.5 (CH_2CHNH), 52.3 (CH_3O), 36.7 (CH_2CHNH), 32.7 (CH_2S), 30.1 (CH_3CO). HRMS calculated for $\text{C}_{21}\text{H}_{22}\text{BrNNaO}_5\text{S}$ $[\text{M} + \text{Na}]^+$ 502.0294, found 502.0291.

(S)-3-(4-((3-Bromobenzyl)oxy)phenyl)-2-(2-mercaptoacetamido)propanoic acid (2j). Off white solid, 0.89 g (70%). ^1H NMR (400 MHz, CD_3OD): δ 7.57 (1H, s, Ar-H), 7.43 (1H, d, $J=8.0$ Hz, Ar-H), 7.37 (1H, d, $J=7.7$ Hz, Ar-H), 7.25 (1H, t, $J=7.8$ Hz, Ar-H), 7.13 (2H, d, $J=8.6$ Hz, Ar-H), 6.89 (2H, d, $J=8.6$ Hz, Ar-H), 5.01 (2H, s, ArCH_2O), 4.61 (1H, dd, $J=8.2$, 5.2 Hz, CH_2CHNH), 3.14 (1H, dd, $J=13.8$, 5.5 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 3.12 (2H, s, CH_2SH), 2.94 (1H, dd, $J=14.0$, 8.0 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$). ^{13}C NMR (100 MHz, CD_3OD): δ 174.3 (CO_2H), 172.9 (CONH), 158.9 (Ar-C), 141.5 (Ar-C), 131.8 (Ar-C), 131.5 (Ar-C), 131.3 (Ar-C), 131.3 (Ar-C), 130.6 (Ar-C), 127.1 (Ar-C), 123.4 (Ar-C), 115.9 (Ar-C), 69.9 (PhCH_2O), 55.3 (CH_2CHNH), 37.5 (CH_2CHNH), 28.0 (CH_2SH). HRMS calculated for $\text{C}_{18}\text{H}_{17}\text{BrNO}_4\text{S}$ $[\text{M}-\text{H}]^-$ 422.0067, found 422.0071.

(S)-Methyl 3-(4-((4-bromobenzyl)oxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoate (5k). Off white solid, 2.90 g (92%). ^1H NMR (500 MHz, CDCl_3): δ 7.48 (2H, d, $J=8.3$ Hz, Ar-H), 7.27 (2H, d, $J=8.3$ Hz, Ar-H), 7.01 (2H, d, $J=8.5$ Hz, Ar-H), 6.85 (2H, d, $J=8.5$ Hz, Ar-H), 4.96 (2H, s, CH_2O), 4.94 (1H, d, $J=7.5$ Hz, NH), 4.52 (1H, q, $J=8.0$ Hz, CH_2CHNH), 3.68 (3H, s, CH_3O), 3.03 (1H, dd, $J=13.8$, 5.8 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 2.97 (1H, dd, $J=13.8$, 6.0 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 1.39 (9H, s, $\text{C}(\text{CH}_3)_3$). ^{13}C NMR (125 MHz, CDCl_3): δ 172.4 (CO_2CH_3), 157.6 (NHCO_2), 155.1 (Ar-C), 136.0 (Ar-C), 131.7 (Ar-C), 130.3 (Ar-C), 129.0 (Ar-C), 128.5 (Ar-C), 121.8 (Ar-C), 114.9 (Ar-C), 79.9 ($\text{C}(\text{CH}_3)_3$), 69.2 (CH_2O), 54.5 (CH_2CHNH), 52.2 (CH_3O), 37.5 (CH_2CHNH), 28.3 ($\text{C}(\text{CH}_3)_3$). HRMS calculated for $\text{C}_{22}\text{H}_{26}\text{BrNNaO}_5$ $[\text{M} + \text{Na}]^+$ 486.0887, found 486.0896.

(S)-Methyl 2-amino-3-(4-((4-bromobenzyl)oxy)phenyl)propanoate (6k). Brown solid, 1.78 g (98%). ^1H NMR (500 MHz, CDCl_3): δ 7.50 (2H, d, $J=8.3$ Hz, Ar-H), 7.30 (2H, d, $J=8.4$ Hz, Ar-H), 7.10 (2H, d, $J=8.5$ Hz, Ar-H), 6.88 (2H, d, $J=8.5$ Hz, Ar-H), 4.96 (2H, s, CH_2O), 3.69 (3H, s, CH_3O), 3.67 (1H, CH_2CHNH_2), 3.01 (1H, dd, $J=13.6$, 5.1 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$), 2.81 (1H, dd, $J=13.7$, 7.7 Hz, $\text{CH}_\text{A}\text{H}_\text{B}\text{CHNH}$). ^{13}C NMR (125 MHz, CDCl_3): δ 175.6 (CO), 157.6 (Ar-C), 136.2 (Ar-C), 131.8 (Ar-C), 130.5 (Ar-C), 129.8 (Ar-C), 129.2 (Ar-C), 122.0 (Ar-C), 115.1 (Ar-C), 69.4 (CH_2O), 56.0 (CH_2CHNH), 52.1 (CH_3O), 40.2 (CH_2CHNH). HRMS calculated for $\text{C}_{17}\text{H}_{19}\text{BrNO}_3$ $[\text{M} + \text{H}]^+$ 364.0543, found 364.0546.

(S)-Methyl 2-(2-(acetylthio)acetamido)-3-(4-((4-bromobenzyl)oxy)phenyl)propanoate (8k). Tan viscous oil, 1.59 g (83%). ^1H NMR

(400 MHz, CDCl₃): δ 7.48 (2H, d, J =8.3 Hz, Ar-H), 7.27 (2H, d, J =8.3 Hz, Ar-H), 6.97 (2H, d, J =8.5 Hz, Ar-H), 6.83 (2H, d, J =8.5 Hz, Ar-H), 6.60 (1H, d, J =7.5 Hz, NH), 4.96 (2H, s, CH₂O), 4.75 (1H, dt, J =7.6, 5.9 Hz, CH₂CHNH), 3.70 (3H, s, CH₃O), 3.51 (2H, dd, J =23.1, 14.8 Hz, CH₂S), 3.06 (1H, dd, J =13.8, 5.5 Hz, CH_AH_BCHNH), 2.97 (1H, dd, J =13.8, 6.1 Hz, CH_AH_BCHNH), 2.31 (3H, s, CH₃CO). ¹³C NMR (100 MHz, CDCl₃): δ 195.2 (C=O), 171.5 (CO₂), 167.6 (CONH), 157.6 (Ar-C), 135.9 (Ar-C), 131.7 (Ar-C), 130.3 (Ar-C), 129.0 (Ar-C), 128.1 (Ar-C), 121.8 (Ar-C), 114.8 (Ar-C), 69.2 (CH₂O), 53.5 (CH₂CHNH), 52.4 (CH₃O), 36.7 (CH₂CHNH), 32.7 (CH₂S), 30.1 (CH₃CO). ⁺HRMS calculated for C₂₁H₂₂BrNNaO₅S [M + Na]⁺ 502.0294, found 502.0289.

(S)-3-(4-((4-Bromobenzyl)oxy)phenyl)-2-(2-mercaptoacetamido)propanoic acid (2k). Off white solid, 0.87 g (68%). ¹H NMR (500 MHz, CD₃OD): δ 7.50 (2H, d, J =8.3 Hz, Ar-H), 7.33 (2H, d, J =8.3 Hz, Ar-H), 7.13 (2H, d, J =8.5 Hz, Ar-H), 6.89 (2H, d, J =8.5 Hz, Ar-H), 5.01 (2H, s, ArCH₂O), 4.61 (1H, dd, J =8.3, 5.3 Hz, CH₂CHNH), 3.14 (1H, dd, J =13.8, 5.5 Hz, CH_AH_BCHNH), 3.12 (2H, s, CH₂SH), 2.94 (1H, dd, J =14.0, 8.0 Hz, CH_AH_BCHNH). ¹³C NMR (125 MHz, CD₃OD): δ 174.4 (CO₂H), 172.9 (CONH), 159.0 (Ar-C), 138.2 (Ar-C), 132.6 (Ar-C), 131.5 (Ar-C), 130.6 (Ar-C), 130.4 (Ar-C), 122.5 (Ar-C), 116.0 (Ar-C), 70.2 (PhCH₂O), 55.3 (CH₂CHNH), 37.5 (CH₂CHNH), 28.0 (CH₂SH). HRMS calculated for C₁₈H₁₇BrNO₄S [M-H][−] 422.0067, found 422.0073.

Note

1. The washing step with water can be reduced to once if freeze-dry equipment is used to evaporate acetic acid but this process can take longer time as the sample should be kept overnight.

Acknowledgements

The authors gratefully acknowledge the assistance of Dr Waleed Hussein, Associate Professor Gerhard Schenk and Associate Professor Ross McGeary at the University of Queensland.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Omid Khalili Arjomandi  <http://orcid.org/0000-0002-1507-1691>
Hadi Adibi  <http://orcid.org/0000-0003-2064-7875>

References

1. Arjomandi OK, Hussein WM, Vella P, et al. Design, synthesis, and in vitro and biological evaluation of potent amino acid-derived thiol inhibitors of the metallo- β -lactamase IMP-1. *Eur J Med Chem* 2016;114:318–27.
2. Yusof Y, Tan DT, Arjomandi OK, et al. Captopril analogues as metallo- β -lactamase inhibitors. *Bioorg Med Chem Lett* 2016; 26:1589–93.
3. King D, Strynadka N. Crystal structure of New Delhi metallo- β -lactamase reveals molecular basis for antibiotic resistance. *Protein Sci* 2011;20:1484–91.
4. Docquier J-D, Mangani S. An update on β -lactamase inhibitor discovery and development. *Drug Resist Update* 2018;36: 13–29.
5. Everett M, Sprynski N, Coelho A, et al. Discovery of a novel metallo- β -lactamase inhibitor that potentiates meropenem activity against Carbapenem-resistant Enterobacteriaceae antimicrob. *Agents Chemother* 2018;62:e00074–18.
6. McGeary RP, Tan DT, Schenk G. Progress toward inhibitors of metallo- β -lactamases. *Future Med Chem* 2017;9:673–91.
7. Drawz SM, Bonomo RA. Three decades of beta-lactamase inhibitors. *Clin Microbiol Rev* 2010;23:160–201.
8. Vella P, Miraula M, Phelan E, et al. Identification and characterization of an unusual metallo- β -lactamase from *Serratia proteamaculans*. *J Biol Inorg Chem* 2013;18:855–63.
9. Hou C-FD, Phelan EK, Miraula M, et al. Unusual metallo- β -lactamases may constitute a new subgroup in this family of enzymes. *J Mol Biol* 2014;04:11–5.
10. Bebrone C. Metallo- β -lactamases (classification, activity, genetic organization, structure, zinc coordination) and their superfamily. *Biochem Pharmacol* 2007;74:1686–701.
11. Crowder MW, Spencer J, Vila AJ. Metallo-beta-lactamases: novel weaponry for antibiotic resistance in bacteria. *Acc Chem Res* 2006;39:721–8.
12. Moran-Barrio J, Gonzalez JM, Lisa MN, et al. The metallo-beta-lactamase GOB is a mono-Zn(II) enzyme with a novel active site. *J Biol Chem* 2007;282:18286–93.
13. Paul-Soto R, Bauer R, Frere J-M, et al. Mono- and binuclear Zn²⁺-beta-lactamase. Role of the conserved cysteine in the catalytic mechanism. *J Biol Chem* 1999;274:13242–9.
14. Phelan EK, Miraula M, Selleck C, et al. Metallo- β -lactamases: a major threat to human health. *J Mol Biol* 2014;4:11–5.
15. Galleni M, Lamotte-Brasseur J, Rossolini GM, et al. Standard numbering scheme for class B β -lactamases. *Antimicrob Agents Chemother* 2001;45:660–3.
16. Lienard BMR, Papamichael C, Schofield CJ, et al. Structural basis for the broad-spectrum inhibition of metallo-beta-lactamases by thiols. *Org Biomol Chem* 2008;6:2282–94.
17. Palzkill T. Metallo- β -lactamase structure and function. *Ann N Y Acad Sci* 2013;1277:91–104.
18. Babini GS, Danel F, Munro SD, et al. Unusual tazobactam-sensitive AmpC β -lactamase from two *Escherichia coli* isolates. *J Antimicrob Chemother* 1998;41:115–8.
19. Herzberg O, Fitzgerald PMD. Metallo β -lactamases. In: *Handbook of metalloproteins*. New York: John Wiley & Sons, Ltd; 2006:217–34.
20. Page MI, Badarau A. The mechanisms of catalysis by metallo beta-lactamases. *Bioinorg Chem Appl* 2008;2008:576297.
21. Díaz N, Suárez D, Merz KM. Zinc metallo- β -lactamase from *Bacteroides fragilis*: a quantum chemical study on model systems of the active site. *J Am Chem Soc* 2000;122:4197–208.
22. Lisa M-N, Palacios AR, Aitha M, et al. A general reaction mechanism for carbapenem hydrolysis by mononuclear and binuclear metallo- β -lactamases. *Nat Commun* 2017;8:538.
23. Ohsuka S, Arakawa Y, Horii T, et al. Effect of pH on activities of novel β -lactamases and β -lactamase inhibitors against these β -lactamases. *Antimicrob Agents Chemother* 1995;39: 1856–8.
24. Goto M, Takahashi T, Yamashita F, et al. Inhibition of the metallo-beta-lactamase produced from *Serratia marcescens* by thiol compounds. *Biol Pharm Bull* 1997;20:1136–40.
25. Mollard C, Moali C, Papamichael C, et al. Thiomandelic acid, a broad spectrum inhibitor of zinc beta-lactamases: kinetic and spectroscopic studies. *J Biol Chem* 2001;276:45015–23.
26. Hammond GG, Huber JL, Greenlee ML, et al. Inhibition of IMP-1 metallo-beta-lactamase and sensitization of IMP-1-

- producing bacteria by thioester derivatives. FEMS Microbiol Lett 1999;179:289–96.
27. Heinz U, Bauer R, Wommer S, et al. Coordination geometries of metal ions in D- or L-captopril-inhibited metallo- β -lactamases. J Biol Chem 2003;278:20659–66.
 28. Hussein WM, Fatahala SS, Mohamed ZM, et al. 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 2012;80:500–15.
 29. Hussein WM, Vella P, Islam NU, et al. 3-Mercapto-1, 2, 4-triazoles and N-acylated thiosemicarbazides as metallo- β -lactamase inhibitors. Bioorg Med Chem Lett 2012;22:380–6.
 30. Mohamed MS, Hussein WM, McGeary RP, et al. Synthesis and kinetic testing of new inhibitors for a metallo- β -lactamase from *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. Eur J Med Chem 2011;46:6075–82.
 31. Simm AM, Loveridge EJ, Crosby J, et al. Bulgecin A: a novel inhibitor of binuclear metallo-beta-lactamases. Biochem J 2005;387:585–90.
 32. Hiraiwa Y, Morinaka A, Fukushima T, et al. Metallo- β -lactamase inhibitory activity of phthalic acid derivatives. Bioorg Med Chem Lett 2009;19:5162–5.
 33. Toney JH, Hammond GG, Fitzgerald PMD, et al. Succinic acids as potent inhibitors of plasmid-borne IMP-1 metallo-beta-lactamase. J Biol Chem 2001;276:31913–8.
 34. Nauton L, Kahn R, Garau G, et al. Structural insights into the design of inhibitors for the L1 metallo- β -lactamase from *Stenotrophomonas maltophilia*. J Mol Biol 2008;375:257–69.
 35. Olsen L, Jost S, Adolph H-W, et al. New leads of metallo- β -lactamase inhibitors from structure-based pharmacophore design. Bioorg Med Chem 2006;14:2627–35.
 36. Feng L, Yang K-W, Zhou L-S, et al. N-Heterocyclic dicarboxylic acids: broad-spectrum inhibitors of metallo- β -lactamases with co-antibacterial effect against antibiotic-resistant bacteria. Bioorg Med Chem Lett 2012;22:5185–9.
 37. Hiraiwa Y, Saito J, Watanabe T, et al. 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 2014;24:4891–4.
 38. Walter MW, Felici A, Galleni M, et al. Trifluoromethyl alcohol and ketone inhibitors of metallo- β -lactamases. Bioorg Med Chem Lett 1996;6:2455–8.
 39. Walter MW, Valladares MH, Adlington RM, et al. Hydroxamate inhibitors of aeromonas hydrophila AE036 metallo-beta-lactamase. Bioorg Chem 1999;27:35–40.
 40. Toney JH, Fitzgerald PMD, Grover-Sharma N, et al. Antibiotic sensitization using biphenyl tetrazoles as potent inhibitors of bacteroides fragilis metallo- β -lactamase. Chem Biol 1998; 5:185–96.
 41. Siemann S, Evanoff DP, Marrone L, et al. N-Arylsulfonyl hydrazones as inhibitors of IMP-1 metallo- β -lactamase. Antimicrob Agents Chemother 2002;46:2450–7.
 42. Büttner D, Kramer JS, Klingler F-M, et al. Challenges in the development of a thiol-based broad-spectrum inhibitor for metallo- β -lactamases. ACS Infect Dis 2018;4:360–72.
 43. González MM, Kosmopoulou M, Mojica MF, et al. Bisthiazolidines: a substrate-mimicking scaffold as an inhibitor of the NDM-1 carbapenemase. ACS Infect Dis 2015;1: 544–54.
 44. Hinchliffe P, González MM, Mojica MF, et al. Cross-class metallo- β -lactamase inhibition by bisthiazolidines reveals multiple binding modes. Proc Natl Acad Sci 2016;113:E3745–54.
 45. Vella P, Hussein WM, Leung EW, et al. The identification of new metallo- β -lactamase inhibitor leads from fragment-based screening. Bioorg Med Chem Lett 2011;21:3282–5.
 46. Laraki N, Franceschini N, Rossolini GM, et al. Biochemical characterization of the *Pseudomonas aeruginosa* 101/1477 metallo- β -lactamase IMP-1 produced by *Escherichia coli*. Antimicrob Agents Chemother 1999;43:902–6.
 47. Bebrone C, Moali C, Mahy F, et al. CENTA as a chromogenic substrate for studying β -lactamases. Antimicrob Agents Chemother 2001;45:1868–71.
 48. Fisher JF, Meroueh SO, Mobashery S. Bacterial resistance to β -lactam antibiotics: compelling opportunism, compelling opportunity. Chem Rev 2005;105:395–424.
 49. Viswanatha T, Marrone L, Goodfellow V, et al. Assays for beta-lactamase activity and inhibition. Methods Mol Med 2008;142:239–60.
 50. Wolf C, Francis CJ, Hawes PA, et al. Enantioselective alkylation of aldehydes promoted by (S)-tyrosine-derived beta-amino alcohols. Tetrahedron: Asymmetry 2002;13:1733–41.
 51. Van Benthem RATM, Hiemstra H, Speckamp WN. Synthesis of N-Boc-protected 1-amino-3-alken-2-ols from allylic carbamates via palladium(II)-catalyzed oxidative cyclization. J Org Chem 1992;57:6083–5.
 52. Knorr R, Trzeciak A, Bannwarth W, et al. New coupling reagents in peptide chemistry. Tetrahedron Lett 1989;30: 1927–30.