Very Important Paper

Rh(II)-Catalyzed De-symmetrization of Ethane-1,2-dithiol and Propane-1,3-dithiol Yields Metallo-β-lactamase Inhibitors

Mikhail Krasavin,[a] Daniil Zhukovsky,[a] Igor Solovyev,[a] Darina Barkhatova,[a] Dmitry Dar’in,[a] Denia Frank,[b] Giada Martinelli,[b] Lilia Weizel,[c] Anna Proschak,[c] Marco Rotter,[c] Jan S. Kramer,[c] Steffen Brunst,[c] Thomas A. Wichelhaus,[b] and Ewgenij Proschak*[c]

Diversity-oriented synthesis (DOS) is a rich source for novel lead structures in Medicinal Chemistry. In this study, we present a DOS-compatible method for synthesis of compounds bearing a free thiol moiety. The procedure relies on Rh(II)-catalyzed coupling of dithiols to diazo building blocks. The synthetized library was probed against metallo-β-lactamases (MBLs) NDM-1 and VIM-1. Biochemical and biological evaluation led to identification of novel potent MBL inhibitors with antibiotic adjuvant activity.

Introduction

Multiresistant ESKAPE (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species) pathogens are a major global threat for human health. Among other resistance factors, β-lactamases are most prevalent and effectively protect the bacteria against the different kind of β-lactam antibiotics, including last resort penems and cephalosporins.[1] The β-lactamase-mediated mechanism of β-lactam hydrolysis relies either on nucleophilic serine residue (in serine β-lactamases, SBLs) or metal ions (in metallo- β-lactamases, MBLs) in the active site of the enzyme. Although in general a β-lactamase inhibitor does not exhibit antimicrobial activity itself, it prevents the rapid degradation of β-lactam antibiotics and thereby acts as antibiotic adjuvant.[2] While SBL inhibitors are widely established, agents inhibiting MBLs or both type of β-lactamases are still under clinical evaluation.[3] Fast evolution of β-lactamases, caused by high selection pressure make the search for new inhibitors highly urgent. One of the possible MBL inhibition mechanism involves inhibitors possessing a thiol moiety. Thiols bind tightly to the Zn²⁺ ions in the MBL active site.[4] Although a great variety of thiol-based inhibitors have been developed which reached very significant binding potency in vitro,[5] none of them reached clinical evaluation yet. Previously we showed that approved drugs exhibiting free thiol moieties potently inhibit different MBLs in vitro.[6] Our efforts to follow the SOSA (selective optimization of side activities) approach[7] to optimize the approved drugs thiorphan and captopril towards efficient MBL inhibitors revealed numerous challenges in the development of thiol-based MBL inhibitors.[8] Recently, we discovered an efficient Rh(II)-catalyzed S–H insertion reaction of α-diazo-γ-butyrolactams with a variety of aromatic and aliphatic thiols.[9] Interestingly, the same reaction with ethane-1,2-dithiol led to the formation of the mono-insertion product in good chemical yield. To the best of our knowledge, the latter reaction represents the first example to a general approach to de-symmetrization of symmetrical aliphatic dithiols 2 using chemistry of diazo compounds 1 (Figure 1). In this article, we describe an application of this novel synthetic approach towards the linking of a thiol moiety to a range of chemically diverse aliphatic scaffolds which led to identification of potent MBL inhibitors among resulting alkylthio-substituted thiols 3 (Figure 1).

[1] Prof. M. Krasavin, D. Zhukovsky, I. Solovyev, D. Barkhatova, Prof. D. Dar’in Institute of Chemistry, Saint Petersburg State University 26 Universitetskii prospect, Peterhof 198905 (Russia) E-mail: m.krasavin@spbu.ru
[b] D. Frank, G. Martinelli, Prof. T. A. Wichelhaus Institute of Medical Microbiology and Infection Control University Hospital Frankfurt Paul-Ehrlich-Straße 40, 60596 Frankfurt (Germany)
[c] L. Weizel, Dr. A. Proschak, M. Rotter, Dr. J. S. Kramer, S. Brunst, Prof. E. Proschak Institute of Pharmaceutical Chemistry Goethe-University Frankfurt Max-von-Laue Str. 9, 60438 Frankfurt a.M. (Germany) E-mail: proschak@pharmchem.uni-frankfurt.de
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Figure 1. De-symmetrization of symmetrical aliphatic dithiols 2 via Rh(III)-catalyzed S–H insertion of diazo compounds 1 exploited in this work.
Results and Discussion

Synthesis of alkylthio-substituted aliphatic thiols 3

The arsenal of diazo compounds 1a–i selected for this study has been reported previously as prepared via the recently developed 'sulfonyl-azide-free' (SAFE) protocol (vide infra).\(^\text{[10–12]}\) \(\alpha\)-Diazo-\(\gamma\)-butyrolactams 1j–n were prepared via the Danheiser diazo transfer protocol using 4-nitrobenzene sulfonyl azide as diazo transfer reagent.\(^\text{[13,14]}\) It should be noted that all aforementioned diazo compounds are stable to storage except for 1j which undergoes a rapid dimerization\(^\text{[14]}\) and had, therefore, been used immediately\(^\text{[13]}\) in the subsequent Rh(II)-catalyzed \(S\)-H insertion reaction without isolation (Figure 2).

In addition to known \(\alpha\)-diazo carbonyl compounds 1a–n, we synthesized a small set of \(\alpha\)-diazo acetamides 1o–r. Amines 4 reacted with 2,2,6-trimethyl-4\(H\)-1,3-dioxin-4-one (5) in refluxing xylene which led to ring opening towards \(\alpha\)-acetyl acetamides 6. The latter, after change of the solvent to acetonitrile, were subjected to the SAFE diazo transfer protocol.\(^\text{[12]}\) After the diazo transfer was complete, brief reaction workup and removal of the acetyl group by treatment with KOH solution in aqueous acetonitrile led to the formation of \(\alpha\)-diazo acetamides 1o–r. The latter were isolated by chromatography in modest yields as calculated over 3 chemical steps (Scheme 1).

With the arsenal of 18 structurally diverse \(\alpha\)-diazo carbonyl compounds 1a–r in hand, we proceeded with coupling the respective Rh(II) carbenes to either ethane-1,2-dithiol or propane-1,3-dithiol, or both, which led to the expected de-symmetrization of the latter and the formation of alkylthio-substituted thiols 3a–x in modest to good yields (Scheme 2).

Biochemical evaluation

In order to investigate the structure-activity relationships of the dithiol library, we measured the inhibitory activity of 3a–x in a fluorescence-based enzyme activity assay (Table 1). Two relevant \(\beta\)-lactamase isoforms, NDM-1 and VIM-1 were selected for in vitro assays. The fluorogenic substrate fluorocillin\(^\text{[15]}\) was prepared according to literature and its conversion was monitored as described previously.\(^\text{[6]}\) In general, a linear relationship between the potency of 3a–x towards both enzymes could be observed, however, inhibitory potency towards VIM-1 was almost tenfold higher (Figure 3). A very clear preference for the propane-1,3-dithiol over ethane-1,2-dithiol compounds could be observed from matched molecular pairs. The most potent compound with a balanced inhibitory activity towards both enzymes was the N-phenyl-\(\gamma\)-lactam derivative.
moiety forms a directed hydrogen bond towards backbone NH responsible for catalytic center. It thereby displaces the polarized water were conducted. Therefore, structures of both possible enantiomers of NDM-1 (PDB code 4EXS) revealed that the free thiol group, which was assumed to be negatively charged, is located between the Zn\(^{2+}\) ions in the catalytic center. It thereby displaces the polarized water responsible for \(\beta\)-lactam hydrolysis. Furthermore, the thioether moiety forms a directed hydrogen bond towards backbone NH of Asn220. The carbonyl oxygen of the cyclopentanone moiety forms a hydrogen bond towards the side chain of Lys211. Both interactions towards Asn220 and Lys211 are described to be important for recognition of the carboxylate moiety of \(\beta\)-lactam.\(^{[17]}\) The binding mode explains the preference of the propane-1,3-dithiol over ethane-1,2-dithiol derivatives, due to the optimal distance of three carbons between the thiol and the thioether groups. (Figure 4)

The docking of the most potent derivative 3o reveals the same preferred interactions as the simplified analogue 3a. (Figure 3B) The N-phenyl ring reaches out to a flat subpocket which is only partially lipophilic and open to solvent. Due to its open nature, a certain variability can be assumed in this area. This hypothesis fits to the observation that various moieties fit this subpocket without significant loss in activity e.g. N-phenyl derivative 3o (IC\(_{50}\)(NDM-1) = 0.3 \(\mu\)M), N-(3-CF\(_{3}\))-phenyl derivative 3q (IC\(_{50}\)(NDM-1) = 0.48 \(\mu\)M), or N-(2-thiazolyl) derivative 3s (IC\(_{50}\)(NDM-1) = 0.39 \(\mu\)M). Notably, only \((S)\)-enantiomers of 3a and 3o were able to display these favourable binding modes while \((R)\)-enantiomers were unable to form all directed interactions in a low-energy conformation. This observation paves the way for future investigations of the enantioselective synthesis route and subsequent biochemical evaluation of the enantiomers.

In order to rationalize the structure-activity relationships of the prepared library, molecular docking experiments with the most potent derivative 3o and the most simple analogue 3a were conducted. Therefore, structures of both possible enantiomers of 3o and 3a were docked into the X-ray structure of NDM-1 (PDB code 4EXS)\(^{[16]}\) in complex with a thiol-containing inhibitor L-captopril. The obtained docking mode of 3a revealed that the free thiol group, which was assumed to be negatively charged, is located between the Zn\(^{2+}\) ions in the catalytic center. It thereby displaces the polarized water responsible for \(\beta\)-lactam hydrolysis. Furthermore, the thioether moiety forms a directed hydrogen bond towards backbone NH of Asn220. The carbonyl oxygen of the cyclopentanone moiety forms a hydrogen bond towards the side chain of Lys211. Both interactions towards Asn220 and Lys211 are described to be important for recognition of the carboxylate moiety of \(\beta\)-lactam.\(^{[17]}\) The binding mode explains the preference of the propane-1,3-dithiol over ethane-1,2-dithiol derivatives, due to the optimal distance of three carbons between the thiol and the thioether groups. (Figure 4)

The docking of the most potent derivative 3o reveals the same preferred interactions as the simplified analogue 3a. (Figure 3B) The N-phenyl ring reaches out to a flat subpocket which is only partially lipophilic and open to solvent. Due to its open nature, a certain variability can be assumed in this area. This hypothesis fits to the observation that various moieties fit this subpocket without significant loss in activity e.g. N-phenyl derivative 3o (IC\(_{50}\)(NDM-1) = 0.3 \(\mu\)M), N-(3-CF\(_{3}\))-phenyl derivative 3q (IC\(_{50}\)(NDM-1) = 0.48 \(\mu\)M), or N-(2-thiazolyl) derivative 3s (IC\(_{50}\)(NDM-1) = 0.39 \(\mu\)M). Notably, only \((S)\)-enantiomers of 3a and 3o were able to display these favourable binding modes while \((R)\)-enantiomers were unable to form all directed interactions in a low-energy conformation. This observation paves the way for future investigations of the enantioselective synthesis route and subsequent biochemical evaluation of the enantiomers.
For further biological evaluation we concentrated on the compounds with the balanced potency towards both, NDM-1 and VIM-1, 3o and 3s. Furthermore, the minimalistic derivative 3a was used for comparison to ensure that the aromatic derivative does not change the mode of action of the compound. Some classes of MBL inhibitors do not directly bind to the active site but act as zinc chelators and withdraw catalytically essential zinc ions.\(^{19}\) We verified the direct inhibitory mode of action by adding 100 \(\mu M\) ZnCl\(_2\) to the recombinant MBL in vitro assay. As Figure 5 shows, addition of zinc ions does not impair the inhibitory activity of 3a, 3o, and 3s, suggesting direct inhibition and binding to the active site.

The next step was the investigation of the binding thermodynamics of 3a, 3o, and 3s. For these experiments we used a closely related enzyme VIM-2 which is an isoform of VIM-1 and can be recombinantly expressed in very high concentrations, suitable for isothermal titration calorimetry (ITC) experiments (Figure 6). ITC titration of 250 \(\mu M\) of 3a, 3o, or 3s, respectively, into 50 \(\mu M\) of VIM-2 revealed potent entropy-driven binding of all three compounds, with 3a being the weakest \((K_d = 0.88 \mu M)\). Notably, binding of 3o displays almost double enthalpy \(\Delta H = -49 \text{ kJ/mol}\) compared to 3a and 3s \((\Delta H = -22 \text{ kJ/mol} \text{ and } -27 \text{ kJ/mol})\).

The considerable inhibition of purified NDM-1 and VIM-1 in vitro suggested that compound 3a, 3o, or 3s, which themselves exhibit no intrinsic antimicrobial activity (Table 2), can potentially restore the activity of imipenem against bacterial isolates producing MBL.

To investigate this, the MIC of imipenem or combined with various concentrations of compound 3a, 3o, or 3s was determined against \(E. coli\) transformants producing NDM-1 or VIM-1 (Table 3 and 4). Especially compound 3o and 3s substantially reduced the MIC of imipenem against NDM-1 or VIM-1 producing bacteria up to 8-fold and 32-fold, respectively.

**Conclusion**

In this study we could show that Rh(II)-catalyzed introduction of dithiols is a highly useful method for diversity-oriented synthesis of chemical libraries which are intended to contain free sulfhydryl groups. We prepared a diverse library of thiol-based inhibitors of MBLs and evaluated them in vitro. Biochemical and biological evaluation of the prepared library showed that potent MBL inhibitors with antibiotic adjuvant activity could be generated.
Experimental Section

Chemical synthesis

General methods

Known diazocarbonyl compounds 1a–n were prepared according to literature procedures,[10–16] other reagents were obtained from commercial sources and used without any additional purification. Solvents were distilled over suitable drying agents. Mass spectra were recorded with a Bruker Maxis HRMS-ESI-qTOF spectrometer (electrospray ionization mode). NMR spectroscopic data were recorded with Bruker Avance 400 spectrometer (400.13 MHz for 1H and 100.61 MHz for 13C) in CDCl3, and were referenced to residual solvent proton peaks (δH = 7.28) and solvent carbon peaks (δC = 77.0). Melting points were determined with a Stuart SMP50 instrument in open capillary tubes.

Preparation of α-diazo acetonamides 1-o-r

A mixture of appropriate amine 4a (1 mmol) and 2,2,6-trimethyl-4H-1,3-dioxin-4-one (5, 1 mmol) in o-xylene (6 mL) was heated at reflux for 1 h and the solution was removed under reduced pressure. The residue was dissolved in acetonitrile (8 mL) and a mixture of 3-(chlorosulfonyl)benzoic acid (292 mg, 1.34 mmol), sodium azide (98 mg, 1.5 mmol) and potassium carbonate (276 mg, 2 mmol) in water (8 mL), pre-stirred over 30 min, was added. The resulting emulsion was stirred for 2 h at room temperature whereupon the diazo transfer was complete. The reaction mixture was extracted with chloroform (2 × 10 mL). The chloroform solution was separated, dried over anhydrous Na2SO4, filtered and concentrated to dryness. The residue was dissolved in acetonitrile (20 mL) and was treated with a solution of KOH (140 mg, 5 mmol) in water (4 mL). The resulting mixture was stirred for 3 h at room temperature and extracted with chloroform (2 × 10 mL). The organic phase was dried over anhydrous Na2SO4, filtered and the solvent was removed under reduced pressure. The residue was purified by column chromatography using ethyl acetate-n-hexane 1:4 as eluent.

2-Diazo-1-(4-tosyl)piperazin-1-yl)ethanone (1t)

Yield 72 mg (24%). Yellow oil. 1H NMR (400 MHz, CDCl3) δ 7.70–7.57 (m, 2H), 7.41–7.34 (m, 2H), 4.94 (s, 1H), 3.52 (s, 4H), 3.01 (t, J = 5.1 Hz, 4H), 2.46 (s, 3H). 13C NMR (101 MHz, CDCl3) δ 164.8, 144.3, 132.0, 129.7, 127.7, 147.5, 43.2 (br s), 21.5. HRMS (ESI-Q/TOF) m/z: [M + Na]+ Calcd for C13H11N4NaO2 246.0405; Found 246.0408.

General procedure for the preparation of compounds 3-a-x

To a vigorously stirred solution of diazo compound 1 (0.5 mmol) and symmetrical dithiol (5 mmol) in dichloromethane (10 mL) an appropriate rhodium(II) catalyst (0.005 mmol of Rh2(OAC)2 for lactams 3-m-t and 0.0025 mmol of Rh2(esp)2 in all other cases) was added. The reaction mixture was stirred at room temperature over 18 h. The volatiles were removed using a rotary evaporator and the desired product was isolated by a column chromatography on silica gel using ethyl acetate–n-hexane 1:4 as eluent.

Preparation of α-diazo acetonamides 1-o-r

Yield 32 mg (97%). Yellow oil. 1H NMR (400 MHz, CDCl3) δ 7.88–7.82 (m, 2H), 7.22–7.15 (m, 2H), 4.89 (s, 1H), 3.87–3.93 (m, 3H), 2.80–2.86 (m, 2H), 2.42 (s, 3H). 13C NMR (101 MHz, CDCl3) δ 164.2, 136.1, 132.0, 130.3, 128.5, 62.1, 52.7, 46.4, 43.8. HRMS (ESI-Q/TOF) m/z: [M + Na]+ Calcd for C13H11N4NaO2 249.0378; Found 249.0388.

Preparation of α-diazo acetonamides 1-o-r

Yield 72 mg (24%). Yellow oil. 1H NMR (400 MHz, CDCl3) δ 7.88–7.82 (m, 2H), 7.22–7.15 (m, 2H), 4.89 (s, 1H), 3.87–3.93 (m, 3H), 2.80–2.86 (m, 2H), 2.42 (s, 3H). 13C NMR (101 MHz, CDCl3) δ 164.8, 144.3, 132.0, 129.7, 127.7, 147.5, 43.2 (br s), 21.5. HRMS (ESI-Q/TOF) m/z: [M + Na]+ Calcd for C13H11N4NaO2 246.0405; Found 246.0408.
Yield 90 mg, 57 %. Yellow oil. 1H NMR (400 MHz, CDCl3) δ 8.28–8.17 (m, 2H), 7.73–7.65 (m, 2H), 4.64 (s, 1H), 4.30–4.16 (m, 2H), 2.76–2.65 (m, 2H), 2.65–2.56 (m, 2H), 1.93–1.82 (m, 2H), 1.34 (t, J = 8.1 Hz, 1H), 1.29 (t, J = 7.1 Hz, 3H). 13C NMR (101 MHz, CDCl3) δ 169.7, 147.7, 143.3, 129.6, 123.9, 62.4, 51.4, 36.0, 24.2, 14.1. HRMS (ESI-Q/TOF) m/z: [M + Na]+ Calcd for C19H14NNaO5S, 324.0345; Found 324.0346.

Ethyl 2-((3-mercaptopropyl)thio)-2-(4-nitrophenyl)thio)acetate (3k)

Yield 122 mg, 73 %. Yellow oil. 1H NMR (400 MHz, CDCl3) δ 7.99–7.81 (m, 2H), 7.54–7.48 (m, 1H), 7.45–7.41 (m, 1H), 4.02 (dt, J = 9.5, 7.4 Hz, 1H), 3.88 (dd, J = 9.5, 8.2, 3.9 Hz, 1H), 3.72 (dd, J = 8.5, 4.5 Hz, 1H), 3.32–3.19 (m, 1H), 3.01–2.91 (m, 1H), 2.90–2.79 (m, 2H), 2.64 (dd, J = 13.5, 8.4, 7.3 Hz, 1H), 2.08 (dd, J = 13.6, 7.6, 4.2 Hz, 1H), 1.75 (t, J = 8.1 Hz, 1H). 13C NMR (101 MHz, CDCl3) δ 172.4, 139.7, 131.3 (q, JNaO 12.9, 7.3 Hz, 1H), 130.9, 128.9, 124.8, 120.0, 46.7, 44.4, 33.2, 29.6, 26.1, 23.5. HRMS (ESI-Q/TOF) m/z: [M + Na]+ Calcd for C17H15NNaO5S, 326.0824; Found 326.0830.

2-Hydroxybenzylidene malonaldehyde (3l)

Yield 96 mg, 52 %. Yellow amorphous solid. 1H NMR (400 MHz, CDCl3) δ 7.68–7.56 (m, 2H), 7.45–7.33 (m, 2H), 7.23–7.11 (m, 1H), 3.97 (dt, J = 9.5, 7.3 Hz, 1H), 3.80 (dd, J = 9.6, 8.2, 4.1 Hz, 1H), 3.65 (dd, J = 8.5, 4.6 Hz, 1H), 1.32–1.28 (m, 2H), 2.88 (dt, J = 12.9, 7.9 Hz, 1H), 2.67 (dt, J = 8.2, 7.0 Hz, 2H), 2.63–2.54 (m, 1H), 2.11–1.92 (m, 1H), 1.43 (t, J = 8.1 Hz, 1H). 13C NMR (101 MHz, CDCl3) δ 167.2, 139.3, 128.9, 124.8, 120.0, 46.7, 44.4, 33.2, 29.6, 26.1, 23.5. HRMS (ESI-Q/TOF) m/z: [M + Na]+ Calcd for C17H15NNaO5S, 326.0824; Found 326.0830.
Yield 77 mg, 59%. Yellow oil. 1H NMR (400 MHz, CDCl₃) δ 7.51 (d, J = 3.6 Hz, 1H), 7.06 (d, J = 3.6 Hz, 1H), 4.29–4.12 (m, 2H), 3.98–3.69 (m, 1H), 3.30–3.14 (m, 1H), 3.05–2.91 (m, 1H), 2.91–2.79 (m, 2H), 2.73–2.65 (m, 1H), 2.31–2.00 (m, 1H), 1.74 (t, J = 8.1 Hz, 1H). 13C NMR (101 MHz, CDCl₃) δ 171.5, 157.6, 137.8, 114.2, 46.0, 43.1, 35.4, 26.2, 24.6. HRMS (ESI-Q/TOF) m/z: [M+Na]+ Calcd for C₁₇H₁₈N₂O₂Na, 261.0185; Found 261.0184.

Yield 50 mg, 36%. Yellow oil. 1H NMR (400 MHz, CDCl₃) δ 7.84–7.77 (m, 2H), 7.72–7.65 (m, 2H), 4.00 (dt, J = 9.6, 7.5 Hz, 1H), 3.84 (dd, J = 9.5, 8.3 Hz, 1H), 3.72 (dd, J = 8.4, 4.3 Hz, 1H), 3.28–3.17 (m, 1H), 2.97–2.90 (m, 1H), 2.90–2.82 (m, 2H), 2.64 (dq, J = 13.6, 8.2 Hz, 1H), 2.09 (ddt, J = 13.6, 7.7, 4.0 Hz, 1H), 1.74 (t, J = 8.1 Hz, 1H). 13C NMR (101 MHz, CDCl₃) δ 172.8, 143.0, 131.1, 119.4, 107.6, 46.3, 44.0, 35.4, 25.7, 24.6. HRMS (ESI-Q/TOF) m/z: [M+Na]+ Calcd for C₁₇H₁₈N₂O₂Na, 297.0160; Found 297.0171.

Yield 122 mg, 68%. Yellow oil. 1H NMR (400 MHz, CDCl₃) δ 7.31 (d, J = 8.3 Hz, 2H), 7.27 (d, J = 8.5 Hz, 2H), 3.63 (t, J = 5.1 Hz, 2H), 3.54–3.45 (m, 4H), 3.30 (s, 2H), 2.77 (t, J = 7.1 Hz, 2H), 2.68–2.60 (m, 2H), 2.47 (t, J = 5.0 Hz, 2H), 2.43 (t, J = 5.2 Hz, 2H), 1.94 (p, J = 7.0 Hz, 2H), 1.39 (t, J = 8.0 Hz, 1H). 13C NMR (101 MHz, CDCl₃) δ 167.6, 136.3, 133.0, 130.3, 128.5, 62.0, 53.0, 52.7, 46.4, 41.8, 33.2, 32.9, 30.5, 23.3. HRMS (ESI-Q/TOF) m/z: [M+Na]+ Calcd for C₁₇H₁₈N₂O₂Na, 301.0440; Found 301.0431.

Yield 28%, 37 mg. Yellow oil. 1H NMR (400 MHz, CDCl₃) δ 4.36–4.22 (m, 0.5H), 3.71–3.41 (2H), 3.41–3.29 (m, 1.5H), 2.99 (s, 1.5H), 2.95–2.83 (m, 3H), 2.83–2.74 (m, 2H), 2.10–2.06 (m, 1H), 1.92–1.67 (m, 4H), 1.63–1.08 (m, 5H). 13C NMR (101 MHz, CDCl₃) δ 170.8, 170.2, 70.5, 69.6, 63.8, 59.3, 36.1, 35.9, 35.2, 34.6, 33.8, 33.5, 30.3, 29.4, 28.5, 27.3, 25.1, 25.0, 24.5, 24.4, 24.3, 24.3. HRMS (ESI-Q/TOF) m/z: [M+H]+ Calcd for C₁₇H₂₀N₄O₅S, 264.1086; Found 264.1095.

Yield 50%, 94 mg. White solid, mp 100.5-101.4 °C. 1H NMR (400 MHz, CDCl₃) δ 7.69–7.58 (m, 2H), 7.38–7.33 (m, 2H), 3.70 (t, J = 5.1 Hz, 2H), 3.58 (t, J = 5.0 Hz, 2H), 3.26 (s, 2H), 3.09 (t, J = 5.0 Hz, 2H), 3.00 (t, J = 5.1 Hz, 2H), 2.84–2.75 (m, 2H), 2.72–2.63 (m, 2H), 2.45 (s, 3H), 1.56 (t, J = 8.0 Hz, 1H). 13C NMR (101 MHz, CDCl₃) δ 167.6, 144.2, 132.3, 129.9, 127.8, 46.0, 45.9, 45.8, 41.1, 36.1, 32.8, 24.2, 21.6. HRMS (ESI-Q/TOF) m/z: [M+H]+ Calcd for C₁₇H₁₆Cl₂O₈S, 375.0865; Found 375.0882.

Biological evaluation

Inhibition of VIM-1 and NDM-1 metallo-β-lactamas

Activity assays for MLBS were performed at room temperature in black polystyrol 96well plates (Corning, Corning, NY, USA) using dicefalotinolidofluorescein (Fluorocillin) as a substrate. Proteins were diluted in assay buffer (HEPES 50 mM, pH 7.5 containing 0.01% Triton X-100), with final protein concentrations of NDM-1: 3 nM, or VIM-1: 4 nM. Samples were supplemented with an equimolar amount of ZnCl₂. An amount of 1 μL of compounds 3a–3x at different concentrations was incubated with 89 μL of enzyme in assay buffer. After an incubation period of 30 min at room temperature, 10 μL of Fluorocillin substrate was added to yield the final assay volume of 100 μL. Final concentration of DMSO was 1%. The fluorescence emitted by the fluorescent product diffusfluor-

Kd determination using isothermal titration calorimetry

VIM-2 expression and purification was published elsewhere.[19] Before the measurement the VIM-2 protein was dialyzed against 500 times the volume of buffer (50 mM Tris, 500 mM NaCl, 5% (v/v) glycerol with a pH = 8) using a dialysis membrane with a 3.5 kDa MWCO. For the measurements the compounds 3a, 3o, or 3s, respectively, were diluted using the dialysis buffer to a final

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concentration of 250 μM containing a final DMSO concentration of 1%. The VIM-2 protein was diluted to a final concentration of 50 μM, supplemented with 1% DMSO using the dialysis buffer and pure DMSO. For the control measurements dialysis buffer was supplemented with pure DMSO to a final concentration of 1%. The measurements were performed using an “Affinity ITC” (TA-Instruments) in reversed mode, with a stir rate of 75 rpm and a temperature of 25°C. The VIM-2 protein was diluted to a final concentration of 250 μM containing a final DMSO concentration of 1%.

Minimal inhibitory concentration determination

Minimal inhibitory concentrations (MICs) of imipenem monohydrate (Sigma-Aldrich) ± compound 3a, 3o, or 3s against transformed E. coli strains producing NDM-1 or VIM-1 were determined according to microdilution method established by Clinical and Laboratory Standards Institute (CLSI). The checkerboard assay was performed to test for synergy in vitro. The microtiter-plates were set up with serial doubling dilutions of compound 3a, 3o, or 3s (2–128 mg/L) and imipenem (0.125–128 mg/L).

Molecular modeling

Docking was performed using MOE2019.0102 (Chemical Computing Group, Montreal, Canada). X-ray structure of NDM-1 (PDB code 3S43) was downloaded from PDB and prepared using QuickPrep routine. Co-crystallized ligand captopril was selected to define the binding site. Induced-fit docking was employed to dock both enantiomers of 3a and 3o, respectively. For initial placement, template “CHCH5…” was used, while rescoring of the obtained conformations was performed by GBVI/WSA dG scoring function to generate 5 low-energy docking poses, which were inspected manually. Poses with the highest score were used for generation of Figure 4.

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Conflict of Interest

The authors declare no conflict of interest.

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