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Thiol-Containing Metallo-β-Lactamase Inhibitors Resensitize Resistant Gram-Negative Bacteria to Meropenem

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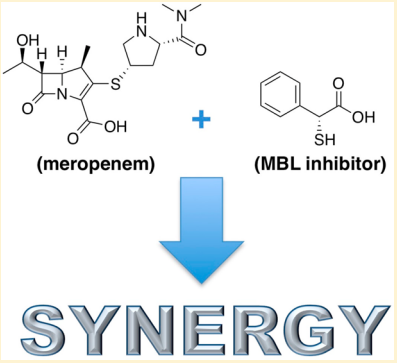
Supporting Information

ABSTRACT: The prevalence of infections caused by metallo-β-lactamase (MBL) expressing Gram-negative bacteria has grown at an alarming rate in recent years. Despite the fact that MBLs can deactivate virtually all β-lactam antibiotics, there are as of yet no approved drugs available that inhibit their activity. We here examine the ability of previously reported thiol-based MBL inhibitors to synergize with meropenem and cefoperazone against a panel of Gram-negative carbapenem-resistant isolates expressing different β-lactamases. Among the compounds tested, thiomandelic acid **3** and 2-mercapto-3-phenylpropionic acid **4** were found to efficiently potentiate the activity of meropenem, especially against an imipenemase (IMP) producing strain of *K. pneumoniae*. In light of the zinc-dependent hydrolytic mechanism employed by MBLs, biophysical studies using isothermal titration calorimetry were also performed, revealing a correlation between the synergistic activity of thiols **3** and **4** and their zinc-binding ability with measured K_d values of 9.8 and 20.0 μM, respectively.



KEYWORDS: *antibiotic resistance, metallo-β-lactamase, MBL inhibitor, zinc binding, synergy*

Resistance to β-lactam antibiotics poses a serious threat to human health. The enzymes responsible for such resistance are the β-lactamases and are especially prevalent among Gram-negative bacteria.[1,2] These enzymes are divided in two classes: the serine β-lactamases (SBLs), which hydrolyze beta-lactam rings by a serine nucleophile in their active site, and the metallo-β-lactamases (MBLs) whose mechanism relies upon the presence of one or two active site zinc ions.[3] These zinc ions stabilize a nucleophilic hydroxide species that is believed to be the active agent in the hydrolysis of the β-lactam ring leading to antibiotic inactivation. The best-studied MBLs include the NDM (New Delhi metallo-beta-lactamase), VIM (Verona integron-encoded metallo-β-lactamase), and IMP (imipenemase) enzymes which collectively exhibit a broad substrate specificity and hydrolyze antibiotics from all known beta-lactam classes.[4]

The global concern relating to antibiotic resistance has led to a number of different strategies aimed at addressing the problem. One such approach involves the coadministration of antibiotic adjuvants capable of maintaining the activity of existing antibiotics.[5] This strategy is widely effective in treating infections due to bacteria that express SBLs. Clinically relevant SBL inhibitors include clavulanic acid, sulbactam, tazobactam, and avibactam which effectively protect β-lactam antibiotics from inactivation when administered as combination therapies. By comparison, there are no clinically used MBL inhibitors available for use in addressing the growing threat posed to the β-lactam arsenal by these enzymes.

Attempts to identify inhibitors of the MBLs have revealed a number of compound classes that display promising activities when tested using *in vitro* enzyme inhibition assays.[6] Such compounds include small molecules that contain functionalities often associated with zinc binding such as thiols, dicarboxylates, hydroxamates, aryl sulfonamides, *N*-arylsulfonyl hydrazones, and tetrazole-based compounds.[3,7,8] Among the known MBL inhibitors, sulfur-containing small molecules containing either a free thiol or a sulfur atom masked as a heterocycle[9,10] are among the best characterized.[3,7,11−15] Structures as simple as mercaptoacetic acid and mercaptopropionic acid have been shown to be effective inhibitors of the IMP-1 enzyme (K_i = 0.23 and 0.19 μM, respectively).[16] Follow-up studies identified higher analogs of mercapto-carboxylic acids including 2-arylmethyl-2-mercaptoacetic acids and their thioesters as potent MBL inhibitors with IC_{50} values in the low-nanomolar range.[17] Similarly, thiomandelic acid was found to be a broad-spectrum inhibitor of different MBLs including BCII (K_i = 0.34 μM), IMP-1 (K_i = 0.029 μM), IMP-2 (K_i = 0.059 μM), and VIM-1 (K_i = 0.230 μM),[18] and 2-ω-phenylpropyl-3-mercaptopropionic acid has been reported as potent inhibitor of VIM-2 (K_i = 0.220 μM).[19,20] Other examples of structurally similar MBL inhibitors include compounds containing a free thiol with a neighboring carbonyl functionality such as α-mercaptoacetophenone,[21] thiosalicylic acid,[18] and interestingly, the dipeptide drug captopril which is used in the treatment of hypertension.[22] The inhibitory activity of these molecules is attributed to the ability of the thiol group to chelate the zinc ion present in the MBL active site as supported by several X-ray crystallography studies.[21,23−26]

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The inhibitory activity of thiol-containing small molecules against clinically relevant MBLs *in vitro* prompted us to conduct a series of antibacterial assays to evaluate the synergistic activity of such compounds with the representative β-lactams meropenem and cefoperazone. To do so, the sensitizing effects of thiols **1**−**5** (Figure 1) on the activity of meropenem and cefoperazone were

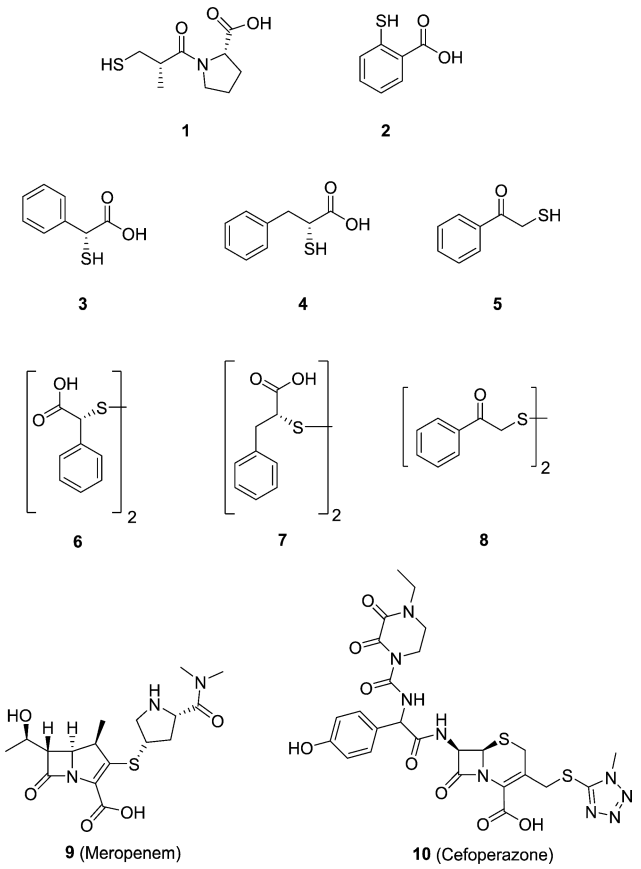

Figure 1. Thiol-based MBL inhibitors and disulfides evaluated for synergy with meropenem and cefoperazone in the current study.

assessed against a panel of Gram-negative bacteria expressing various β-lactamases. The stability of the thiols was also assessed under the assay condition employed, and isothermal titration calorimetry (ITC) was used to measure the zinc-binding affinity of the most synergistically active compounds.

RESULTS AND DISCUSSION

Thiols **1**−**5** were initially tested alone for antibacterial activity against a panel of carbapenem-resistant Gram-negative pathogens expressing MBLs including NDM, VIM, and IMP or SBLs such as KPC-2 and OXA-48. These studies revealed that none of the thiols inhibited bacterial growth at the highest concentration tested of 64 μg/mL. With the exception of *P. aeruginosa* M-120, which was susceptible to meropenem, all of the MBL-expressing strains used in our study exhibited resistance to both meropenem and cefoperazone with MIC values ranging from 8 to >256 μg/mL. For use as a reference MBL inhibitor known to synergize with β-lactam antibiotics, we turned to the work of Migliavacca and co-workers who reported a zinc chelating mixture of EDTA and 1,10-phenanthroline as being synergistic with imipenem to prevent growth of MBL-expressing strains of *Pseudomonas aeruginosa*.[27] We found that the EDTA/1,10-phenanthroline mixture was similarly effective in lowering the MIC of meropenem and cefoperazone against the MBL-expressing strains used in our study (Table 1). Also, and as expected, the EDTA/1,10-phenanthroline mixture showed no synergistic effect with meropenem against the two SBL-expressing strains also evaluated.

With a reliable reference system in hand, the capacity for thiols **1**−**5** to synergize with meropenem was next investigated. Table 1 shows the synergy data of the two most potent thiols **3** and **4** (see Table S2 for complete activity data for compounds **1**−**5**). Captopril **1** exhibited moderate to weak synergy at a concentration of 64 μg/mL while thiosalicylic acid **2** displayed no appreciable synergy when tested at the same concentration. By comparison, when administered at 64 μg/mL, thiols **3** and **4** significantly lowered the MIC of meropenem against all the MBL-producing isolates tested. The activity of compound **5** was also promising but interestingly was limited to only the two *Klebsiella* strains tested. Thiols **3**−**5** have previously been shown to be more potent MBL inhibitors than compounds **1** and **2** in biochemical enzyme inhibition assays,[17,18,21,23] and our MIC synergy results follow the same trend. Notably, for compounds **3** and **4**, we observed broad-spectrum and, in some cases, potent synergistic activity with meropenem against the MBL-producing isolates evaluated. Building on the encouraging results of the preliminary synergy assays (carried out at fixed thiol concentration of 64 μg/mL), we next performed a series of checkerboard synergy assays in which the MIC of meropenem was determined at varying concentrations of inhibitors **1**−**5**. Such an approach provides for a better picture of the synergistic relationship between the two combined agents and allows for determination of the fractional inhibitory concentration (FIC) index. Briefly, FIC values are calculated by adding the following two fractional values: (MIC of compound A in combination/MIC of compound A alone) + (MIC of compound B in combination/MIC of compound B alone). In general, an FIC index value <0.5 is regarded as an indication of synergy.[28] A complete overview of all the checkerboard assays performed as well as the corresponding FIC index values is provided in the Supporting Information. Among the MBL-expressing strains used, the two *Klebsiella* isolates were most effectively resensitized to the meropenem when administered in combination with thiols **3**−**5**. Of particular note, compounds **3** and **4** were both found to significantly potentiate meropenem against the IMP-28 producing *Klebsiella* strain tested with FIC values $\leq$0.07 and $\leq$0.13, respectively (based on the concentrations tested; see Table 1 for checkerboard FIC data of thiols **3** and **4** and Supporting Information for graphical representation of checkerboard assays).

Thiols are well-known for their tendency to form homo- or heterodisulfides in biological systems. Such reactivity is of special importance in the case of thiol-based MBL inhibitors such as compounds **1**−**5** as it has been reported that in their disulfide form their activity is significantly reduced.[18] In this regard, we selected compounds **3**−**5** as the three most active thiols from our synergy assays and monitored their conversion to the corresponding disulfides under the assay conditions used. Thiols **3**−**5** were thus incubated in Mueller-Hinton broth at 37 °C, and sample aliquots were analyzed at time points ranging from 0 to 8 h. As shown in Figure 2, thiols **3** and **4** were found to form their corresponding disulfides (**6** and **7**, respectively) with half-lives of *ca.* 5 h. By comparison, thiol **5** was oxidized to **8** more rapidly with a half-life in the range of minutes which may also explain its lower level of synergy relative to **3** and **4**. Disulfides **6**−**8** were synthesized for use as reference compounds in the stability assays

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Table 1. MIC (μg/mL) of Meropenem (Mer) and Ceferazone (Cef) Tested Alone or in Combination with Thiol MBL-Inhibitors 3 and 4

isolates	Mer	Cef	Mer + 3[a]	Cef + 3	Mer + 4	Cef + 4	Mer + EP[b]	Cef + EP[b]
K. pneumoniae RC10 (KPC-2)	>128	>256	128	>256	>128	>256	>128[c]	>256[c]
K. pneumoniae RC 45 (OXA-48)	64	>256	64	>256	64	>256	16[d]	>256[d]
K. pneumoniae RC51 (VIM-1)	64/32	>256	0.5 (**≥128**,[e] **≤0.27**[f])	256	1 (**32**,[e] **≤0.38**[f])	>256	≤0.5[c]	8[c]
K. pneumoniae JS265 (IMP-28)	16/8	256	0.125 (**128**,[e] **≤0.07**[f])	≤2 (**≥128**,[e] **≤0.07**[f])	0.125 (**64**,[e] **≤0.13**[f])	≤2 (**≥128**,[e] **≤0.07**[f])	≤0.125[g]	≤2[g]
E. coli RC89 (NDM-1)	128/64	>256	16 (**8**[e])	>256	16 (**8**[e])	>256	≤1[c]	>256[c]
P. aeruginosa RC60 (VIM)	32	128	4 (**8**,[e] **≤0.38**[f])	16 (**8**,[e] **≤0.5**[f])	8 (4[e])	16 (**8**,[e] **≤0.5**[f])	0.5[c]	8[c]
P. aeruginosa JS80 (IMP)	64	256	8 (**8**[e])	8 (**32**,[e] **≤0.38**[f])	8 (**8**,[e] **≤0.5**[f])	16 (**16**,[e] **≤0.25**[f])	4[g]	4[g]
*K. pneumoniae*RC21 (VIM-1)	64/32	>256	0.5 (**64**,[e] **≤0.19**[f])	>256	1 (**32**,[e] **≤0.38**[f])	>256	≤0.5[g]	≤2[g]
E. aerogenes RC22 (VIM-1)	32/16	>256	0.5 (**64**,[e] **≤0.25**[f])	>256	1 (**16**,[e] **≤0.38**[f])	>256	≤0.25[g]	64[g]
*K. pneumoniae*RC48 (VIM-1)	>128/128	>256	4 (**>32**,[e] **≤0.38**[f])	>256	8 (**16**,[e] **≤0.5**[f])	>256	≤1[g]	≤2[g]
K. pneumoniae JS22 (NDM-1)	32/16	>256	8 (4[e])	>256	2 (**16**,[e] **≤0.38**[f])	>256	≤0.5[g]	>256[g]
K. pneumoniae JS177 (NDM-1)	16/8	>256	4 (4[e])	>256	1 (**8**[e])	>256	≤0.25[g]	>256[g]
K. pneumoniae JS37 (NDM-1)	64/32	>256	16 (4[e])	>256	16 (4[e])	>256	≤1[g]	>256[g]
P. aeruginosa M-120 (IMP)	<1	128/64	n.d.	2 (**32**,[e] **≤0.25**[f])	n.d.	4 (**16**,[e] **≤0.38**[f])	n.d.	4 (32)[d]

[a]Thiols **3** and **4** added at 64 μg/mL. None of the thiols inhibited growth at this concentration when tested alone. [b]EP = EDTA/1,10-phenanthroline mixture. [c]EP mixture applied at 16 μg/mL EDTA and 1 μg/mL 1,10-phenanthroline. [d]EP mixture applied at 64 μg/mL EDTA and 4 μg/mL 1,10-phenanthroline. [e]Fold reduction of MIC. Reduction of MIC greater than 8-fold shown in bold. [f]Lowest FIC measured (FIC < 0.5 regarded as an indication of synergy). [g]EP mixture applied at 32 μg/mL EDTA and 2 μg/mL 1,10-phenanthroline.

and were evaluated for their synergy with meropenem against the two most susceptible *Klebsiella* isolates identified (Table S20). The three disulfides exhibited very low levels of synergy relative to that of the corresponding free thiols. The slight synergy observed for these disulfides may in fact be attributable to a reductive process carried out by the bacteria themselves to release a small amount of the more active thiol. Many bacteria contain redox active enzymes capable of disulfide reduction both in cytoplasm and periplasmic space.[29]

The superior synergistic activity and relative stability of thiols **3** and **4** prompted us to further characterize their zinc binding abilities using isothermal titration calorimetry (ITC). To do so, a solution of zinc chloride was titrated into the sample well containing either **3** or **4** (both **3** and **4** were both to be found stable in the buffer conditions used for the ITC experiments) and the heat of binding was monitored. In this way, a number of thermodynamic binding parameters are revealed including K_d (dissociation constant), ΔH (enthalpy), ΔG (Gibbs free energy), and ΔS (entropy). As shown in Figure 3, compounds **3** and **4** exhibited high affinities for Zn^{2+} with K_d values of 9.8 and 20.0 μM, respectively. Also of note was the lack of any measurable binding interaction when zinc chloride was titrated into solutions of disulfides **6** and **7**. The zinc binding abilities of the reference compounds EDTA and 1,10-phenanthroline were also assessed using ITC showing a strong interaction (Figures S3 and S4). The results of these ITC studies correlate well with the synergy data obtained and suggest that zinc binding may be a useful predictor for a compound's ability to resensitize MBL-expressing organisms to beta-lactam antibiotics. Furthermore, the relative ease with which ITC can be used to assess zinc binding by small molecules may make it a complementary technique for identifying new lead compounds capable of effectively inhibiting MBLs.

CONCLUSION

While small molecule thio-carbonyl compounds have previously been shown to inhibit various MBLs, their ability to synergize with β-lactam antibiotics in overcoming MBL-associated resistance has not been extensively studied. We here demonstrate a significant level of synergism between meropenem and a series of thiols, most notably thiomandelic acid **3** and 2-merpto-3-phenylpropionic acid **4**. Combinations of meropenem with **3** or **4** exhibit antibacterial activity against a number of Gram-negative bacteria expressing different MBLs including IMP, NDM, and VIM. Given the high degree of active site heterogeneity among the different types of MBL enzymes,[11] designing an inhibitor with potent inhibitory activity toward several types of MBL is challenging. In this light, thiomandelic acid **3** is unique given its ability to inhibit a range of MBLs and, as shown in the present study, its capacity to resensitize MBL-expressing Gram-negative isolates to meropenem, an important β-lactam antibiotic of last resort. In addition, ITC studies showed thiols **3** and **4** to be effective zinc chelators with low-micromolar K_d values supporting the proposed mechanism of action for these compounds. In this regard, ITC may provide a useful means of (pre)screening for zinc-binding MBL inhibitors. While compounds **3** and **4** exhibit potent synergy with meropenem, their propensity to oxidize and likely ability to interact with free zinc and other metallo proteins precludes their use as clinical MBL inhibitors. In this regard, MBL inhibitors employing free thiols as zinc chelating groups are more likely to be of value as tool compounds for biochemical studies involving MBLs. Optimized analogues or other classes of MBL inhibitors capable of overcoming such pharmacokinetic hurdles present a key objective in the continued fight against antibiotic resistance.

MATERIALS AND METHODS

Materials. Potassium thioacetate was purchased from Combi-Blocks Inc. (San Diego, CA USA). Thiosalicylic acid was purchased from Fisher Scientific (Waltham, MA USA). Other reagents, including captopril, 2-bromoacetophenone, *S*-mandelic acid, and L-phenylalanine, were purchased from Sigma-Aldrich company.

Synthesis. Among the thiols selected for investigation, captopril **1** and thiosalicylic acid **2** were commercially available while compounds **3**−**5** required preparation via previously reported synthetic routes.[21,30−32] Briefly, compound **3** was synthesized via esterification of *S*-mandelic acid which was

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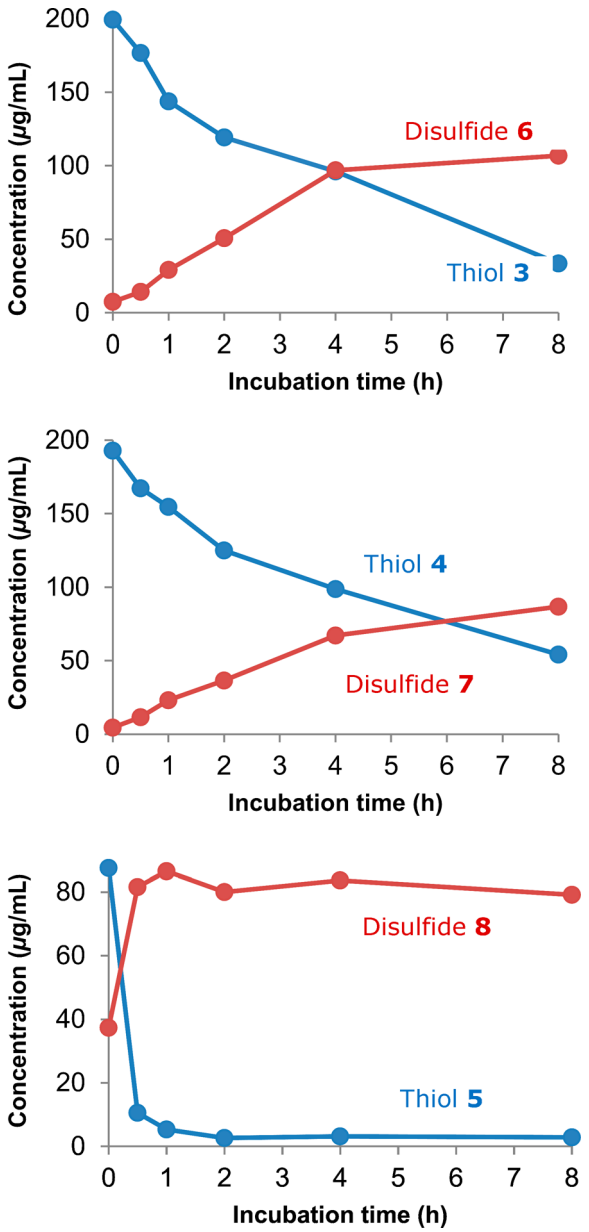

Figure 2. Time-dependent oxidation of thiols **3**−**5** to corresponding disulfides **6**−**8** by incubation in Mueller-Hinton broth at 37 °C.

followed by mesylation of the hydroxyl group. Substitution of the tosylate ester with thioacetate anion followed by acidic hydrolysis furnished **3**.[30] For the synthesis of compound **4**, L-phenylalanine was converted to its corresponding α-bromocarboxylic acid through a sodium nitrite mediated halo-deamination reaction, which was subsequently reacted with potassium thioacetate to afford S-acetyl derivative of **4**. Basic hydrolysis of the latter intermediate led to the final product **4**.[31,32] Thioacetophenone **5** was prepared via a two-step procedure involving thiolation of α-bromoacetophenone with potassium thioacetate followed by a basic hydrolysis.[21] In addition, disulfides **6**−**8**[33−35] were readily prepared by reacting the corresponding thiol with iodine in water/acetonitrile.[36]

MIC Determinations and Synergy Assays. The antibacterial activity of compounds **1**−**5** was evaluated alone and in combination with meropenem against a panel of β-lactamase producing Gram-negative bacteria including *K. pneumoniae* RC10 (KPC-2), *K. pneumoniae* RC 45 (OXA-48), *K. pneumoniae* RC51 (VIM-1), *K. pneumoniae* JS265 (IMP-28), *E. coli* RC89 (NDM-1), *P. aeruginosa* RC60 (VIM), *P. aeruginosa* JS80 (IMP), *K. pneumoniae* RC21 (VIM-1), *E. aerogenes* RC22 (VIM-1), *K. pneumoniae* RC48 (VIM-1), *K. pneumoniae* JS22 (NDM-1), *K. pneumoniae* JS177 (NDM-1), *K. pneumoniae* JS37 (NDM-1), and *P. aeruginosa* M-120 (IMP). The CLSI guidelines were used to determine minimum inhibitory concentrations (MICs). Starting from glycerol stocks, bacterial strains were cultured on blood agar plates and incubated at 37 °C. A single colony was then transferred to tryptic soy broth (TSB) and incubated with shaking at 37 °C until the optical density of the bacterial suspension reached a level equivalent to the 0.5 McFarland standard. The suspension was then diluted to 10^6 CFU/mL in Mueller-Hinton broth (MHB). Using polypropylene microtiter plates, the wells of the first row received 50 μL of the test compounds dissolved in MHB and were subjected to serial dilution. Finally, 50 μL of the bacterial suspension was added, and the plates were sealed and incubated at 37 °C with constant shaking (at 600 rpm). The next morning, the plates were inspected for visible bacterial growth.

Synergy between meropenem (or cefoperazone) and thiols **1**−**5** was evaluated as follows: To the top row of a 96 well plate, 100 μL of a solution of meropenem at 4× MIC was added. 50 μL aliquots of this solution were serially diluted down each row to achieve a range of decreasing meropenem concentrations. Next, thiols **1**−**5** (50 μL aliquots) were added to the wells to provide a concentration range of 128 to 16 μg/mL. The relevant bacterial suspension (100 μL/well) was finally added to each well to give a final concentration of thiols **1**−**5** ranging from 64 to 8 μg/mL. The plates were sealed and incubated overnight at 37 °C. MICs were determined the next morning by visual inspection and used in calculating fractional inhibitory concentration index (FICI) values. As a standard MBL inhibitor cocktail, a 16:1 (w/w) mixture of ethylenediaminetetraacetic acid disodium salt-phenanthroline (EP) was also used. The MIC of the EP mixture was first determined against each strain so that sub-MIC concentrations could be used for synergy assays. All the assays were performed in duplicates. Tables S3−S17 provide a graphical representation of the checkerboard assays.

Stability Analysis. To prepare calibration curves for thiols **3**−**5** and the corresponding disulfides **6**−**8**, each compound was dissolved in DMSO to prepare a stock solution (10 mg/mL) that was then immediately diluted in Mueller-Hinton broth (MHB) to reach concentrations ranging from 1 to 256 μg/mL. The samples were then processed as follows: To precipitate undesired media components, the sample solutions were diluted with acetonitrile (1:3 v/v), vortexed for 10 s, and centrifuged at 10 000 rpm for 10 min. The supernatant was retained and stored at −20 °C until HPLC analysis. Samples were analyzed by analytical RP-HPLC using a Phenomenex Gemini C-18 110A column (250 × 4.60 mm, 5 μm) at a flow rate of 1 mL/min, and their UV absorbances were detected at 214 nm. For the analysis of compounds **4**, **5**, **7**, and **8**, the gradient started with 0% buffer B (95% acetonitrile, 0.1% TFA) and 100% buffer A (5% acetonitrile, 0.1% TFA) increasing to 50% buffer B over 5 min followed by an increase to 100% buffer B over 10 min and maintenance at 100% buffer B for 3 min. The buffer gradient was then returned to 0% buffer B in 2 min and maintained at 0% for an additional 5 min to re-equilibriate the system. For the analysis of compounds **3** and **6**, the gradient started with 0% of buffer B, increased to 50% buffer B over 5 min, and then to 90% buffer B over 8 min followed by a final increase to 100% buffer B over 1 min. After 1 min at 100% buffer B, the gradient returned to 0%

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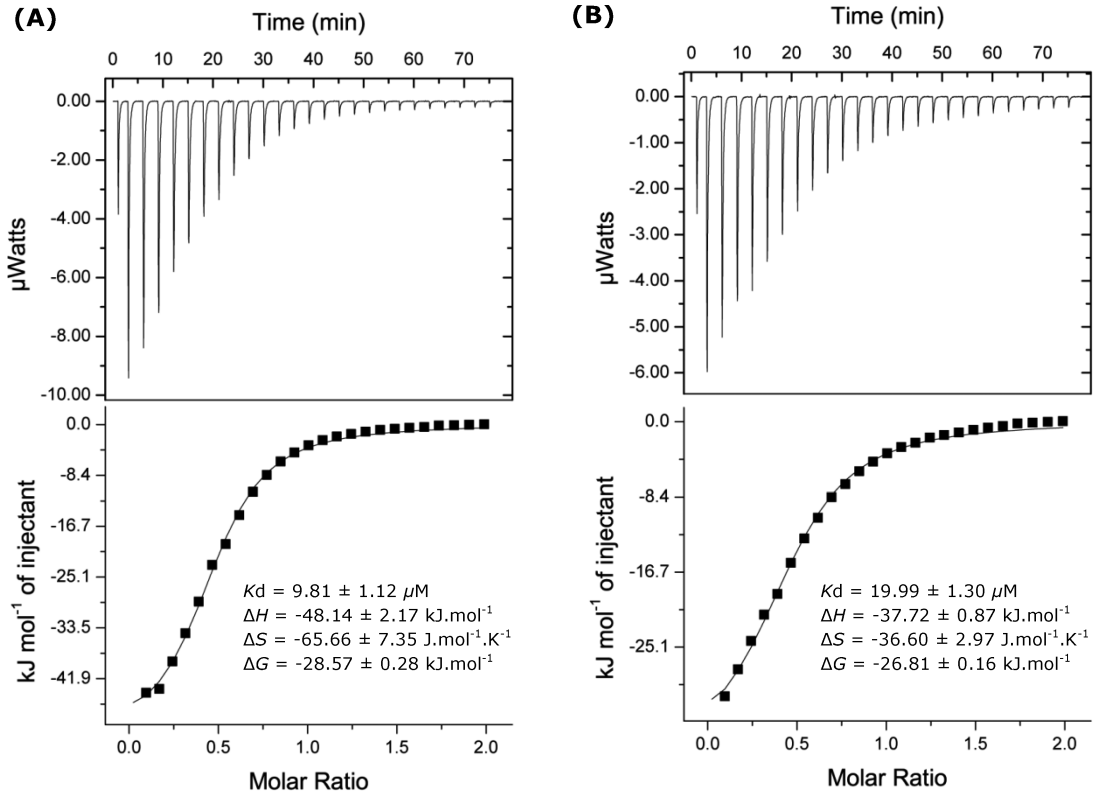

Figure 3. ITC thermograms for binding of Zn^{2+} by thiols **3** (A) and **4** (B). A solution of zinc chloride (2.0 mM) was titrated into the sample cell containing thiol **3** or **4** (0.2 mM). Thermodynamic parameters shown based on triplicate binding assays and reported as mean ± SE. Errors estimated via Monte Carlo simulations using the error of individual experiments.

buffer B over 2 min and was maintained at 0% for 3 min to re-equilibriate the system. The calibration curves were linear from 2 to 256 μg/mL for compounds **3**−**6** and from 1.6 to 200 μg/mL for compounds **7** and **8** with $r^2 > 0.990$ in all the cases. Due to the relatively short half-life of compound **5** in MHB, the medium needed to be supplemented with 5.0 mM TCEP to obtain a suitable calibration curve.

To assess the half-lives of thiols **3**−**5** in MHB, each compound was dissolved in MHB (200 μg/mL) and incubated at 37 °C. At time points of 0, 0.5, 1, 2, 4, and 8 h, 100 μL aliquots of each sample were taken and subjected to the same processing described above for the standards prior to HPLC analysis.

Isothermal Titration Calorimetry. ITC experiments were performed using a MicroCal Auto-ITC200 instrument (Malvern). The test compounds and zinc chloride were dissolved in Tris−HCl buffer (20 mM, pH 7.0) and degassed using a sonication bath (10 min) before running the experiments. The zinc chloride solution was titrated into a solution of **3**, **4**, **6**, **7**, EDTA, or 1,10-phenanthroline (see Table 2 for specific concentrations used) over 26 aliquots of 1.5 μL (except the first injection which was 0.5 μL) with 120 s between injections.

Table 2. Concentrations of the Metal/Ligands Used for the ITC Experiments

experiment (metal/ligand)	zinc chloride concentration (mM)	ligand concentration (mM)
$ZnCl_2$/compound **3**/**4**/**6**/**7**	2.0	0.2
$ZnCl_2$/EDTA	0.4	0.04
$ZnCl_2$/1,10-phenanthroline	1.0	0.1

All the experiments were performed at 25 °C in triplicate with reference power set at 2.0 μcal/s. The generated peaks were integrated using Origin 7.0 software. The error for all the reported thermodynamic parameters was estimated through Monte Carlo simulation with the standard errors of three experiments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsinfecdis.7b00094.

Spectral characterization of compounds **3**−**8** including ^{1}H NMR, ^{13}C NMR, and high-resolution mass spectrometry data; tables of antibiotic susceptibility and checkerboard synergy assays using meropenem or cefoperazone combined with thiols **1**−**5** or disulfides **6**−**8** (Tables S1−S20); ITC thermograms of binding interactions between Zn^{2+} and thiols **3** and **4**, disulfides **6** and **7**, 1,10-phenanthroline, and EDTA (Figures S1−S9) (PDF)

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Notes

The authors declare no competing financial interest.

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