ACS Medicinal Chemistry Letters

Letter

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Carbamylmethyl Mercaptoacetate Thioether: A Novel Scaffold for the Development of L1 Metallo-β-lactamase Inhibitors

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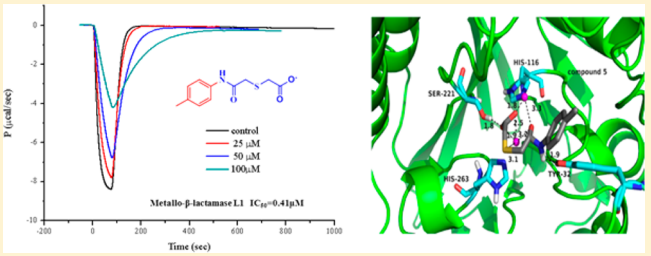
Supporting Information

ABSTRACT: Given the clinical importance of metallo-β-lactamases (MβLs), a new scaffold, N-substituted carbamylmethyl mercaptoacetate thioether, was constructed. The obtained molecules **1**−**16** inhibited MβLs from all three subclasses, but preferentially L1 from subclass B3. Compound **9** with a *p*-carboxyphenyl substituent exhibited the broadest spectrum with at least 70% inhibition of enzymes from all subclasses at 100 μM, while compound **5** with a *p*-methylphenyl substituent was the most potent inhibitor of any individual enzyme, with 97% inhibition at 100 μM and an IC_{50} value of 0.41 μM against L1. Isothermal titration calorimetry assays corroborate findings from UV−vis spectrophotometric assays that the inhibition of L1 by **5** is dose-dependent. Docking studies suggest that the carboxyl group, the sulfide atom, and the carbonyl group of the carbamyl coordinate Zn2 in a chelating fashion. Using *E. coli* cells expressing L1, **6** and **8** were able to decrease cefazolin minimum inhibitory concentration 8-fold.



KEYWORDS: *Antibiotic resistance, metallo-β-lactamase, L1, inhibitor, mercaptoacetate thioether*

Antibiotic resistance has become a concerning global health problem,[1] with the World Health Organization (WHO) reporting "that several million people are infected with antibiotic-resistant bacteria per annum."[2] β-Lactam antibiotics are the most broadly prescribed drugs for the treatment of bacterial infections, but their effectiveness has been threatened by the emergence of pathogenic bacteria producing β-lactamases,[3] which catalyze β-lactam hydrolysis.[4] β-Lactamases have been categorized as serine-β-lactamases (SβLs, Ambler classes A,[5] C, and D) and metallo-β-lactamases (MβLs, Ambler class B), according to their mechanisms of action. MβLs are further divided into three subclasses B1, B2, and B3, based on their amino acid sequence and metal occupancies.[6,7]

Since MβLs can deactivate most β-lactam antibiotics, including widely used drug families, such as penicillins, cephalosporins, and carbapenems,[8,9] the development of inhibitors of MβLs is an essential strategy for maintaining the usefulness of existing β-lactam antibiotics. In combination with appropriate antibiotics, clavulanic acid, tazobactam, sulbactam, and avibactam[10,11] have been widely approved for clinical treatment of antibiotic-resistant bacteria containing SβLs. Meanwhile, various MβL inhibitors have been reported,[12] including trifluoromethyl alcohol and ketone,[13] dicarboxylic acids,[14,15] thiols,[16] sulfates,[17] hydroxamates,[18] tetrazoles,[19] and sulfonamides.[20] However, there are no reports of clinically useful MβL inhibitors.[21] Accordingly, the development of new MβL inhibitor scaffolds is urgently needed.[22]

It has been reported that a series of mercaptoacetic acid thioester derivatives inhibited MβLs.[23] Recently, our studies indicated that the amino acid mercaptoacetic acid thioester is a highly promising scaffold for the development of MβL L1 (subclass B3) inhibitors, exhibiting IC_{50} values in the submicromolar range using cefazolin as substrate, and partial thioesters also showed effective inhibitory activities against the MβLs NDM-1 (subclass B1) and ImiS (subclass B2).[24] Docking studies revealed potential binding modes of the amino acid thioesters to the active site of L1 in which the carboxylate group interacts with both Zn(II) ions and Ser221, and the acylamino group forms hydrogen bonds with Tyr32.[24] That scaffold was subsequently optimized to yield more potent L1 inhibitors.[25] Also, our studies revealed that azolylthioacetamides with an acylamino group are potential broad-spectrum inhibitors of MβLs.[26,27]

The earlier mentioned amino acid mercaptoacetic acid thioesters were potent inhibitors of L1, a subclass B3MβL, but were less potent against other, more clinically important MβLs

Received: February 14, 2017
Accepted: April 24, 2017
Published: April 24, 2017

DOI: 10.1021/acsmedchemlett.7b00058
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

tested. In addition, there is a possibility that thioesters could be hydrolyzed and yield mercaptoacetate, which could inhibit other metalloenzymes and thus cause toxicities. In this work, we therefore constructed a novel series of compounds containing a thioether rather than a thioester, the N-substituted carbamylmethyl mercaptoacetate thioethers. The novel molecules were found to be significantly more stable when incubated in aqueous solutions of pH 5.0, 7.0, and 9.0 over a period of 4 days than the previously reported thioesters, as monitored by UV−vis spectra (Figure S1). These new molecules were tested against various MβLs, indicating preference for L1. Inhibition of L1 was investigated in more detail by steady-state kinetics and isothermal titration calorimetry (ITC). Cytotoxicity of the compounds against L-929 mouse fibroblastic cells was tested, their potential interactions with the enzyme were investigated by molecular docking, and their ability to inhibit L1 in *E. coli* cells was tested by determining cefazolin minimum inhibitory concentrations (MICs).

Sixteen N-substituted carbamylmethyl mercaptoacetate thioethers were synthesized by a synthetic route shown in Scheme S1 in the Supporting Information. In brief, amine was added to a mixture of dichloromethane and triethylamine with stirring on an ice−water bath, chloroacetyl chloride was dropwise added, and the resulting mixture was stirred at room temperature for 2 h. The solvent was removed under vacuum, and the resulting residue was recrystallized with anhydrous ethanol to offer 2-chloro-N-substituted carbamylmethyl chloride. Mercaptoacetic acid was dripped into a solution of acetone with KOH, the resulting mixture was added into a solution of the intermediate N-substituted carbamylmethyl chloride dissolved in acetone and refluxed for 2 h at 60 °C. After cooling to room temperature, the precipitate formed was filtered off, washed with anhydrous ethanol, and dried under vacuum to give the target compounds. The structures of the N-substituted carbamylmethyl mercaptoacetate thioethers are shown in Figure 1. All compounds were characterized by ^{1}H and ^{13}C NMR and confirmed by MS (see Supporting Information).

To test whether these compounds were inhibitors of MβLs, several enzymes were overexpressed and purified as previously described[28] and are detailed in the Supporting Information. The inhibitory activities of the prepared carbamylmethyl mercaptoacetate thioethers against purified MβLs from different subclasses were tested on an Agilent UV8453 UV−vis spectrophotometer using 50 μM cefazolin as substrate for B1 and B3 enzymes and 40 μM imipenem for ImiS (B2) and 100 μM inhibitor in the enzyme-specific buffer. Enzyme and inhibitor were preincubated

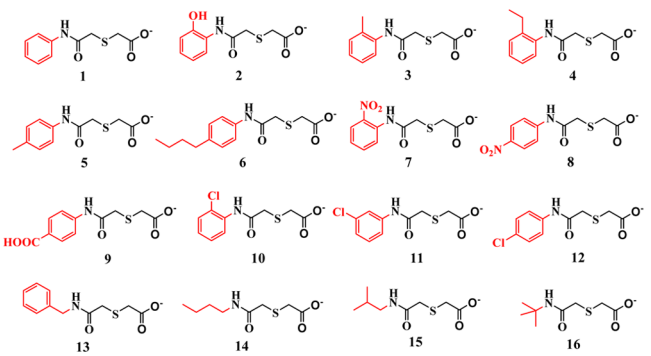


Figure 1. Structures of the synthesized N-substituted carbamylmethyl mercaptoacetate thioethers.

Table 1. Percent Inhibition of IMP-1, ImiS, and L1 by 100 μM N-Substituted Carbamylmethyl Mercaptoacetate Thioether[a]

	% inhibition				% inhibition		
compd	IMP-1	ImiS	L1	compd	IMP-1	ImiS	L1
1	43	18	92	**9**	71	87	75
2	40	74	95	**10**	53	39	80
3	50	24	95	**11**	48	34	79
4	80	21	92	**12**	48	25	73
5	37	52	97	**13**	51	21	97
6	64	29	97	**14**	55	20	89
7	44	21	77	**15**	59	23	78
8	45	85	71	**16**	69	21	91

[a]The substrate for IMP-1 and L1 was cefazolin, and for ImiS, it was imipenem.

Table 2. IC_{50} of N-Substituted Carbamylmethyl Mercaptoacetate Thioethers against L1[a]

compd	IC_{50} (μM)	compd	IC_{50} (μM)
1	0.98 ± 0.04	**9**	7.5 ± 0.6
2	0.58 ± 0.02	**10**	1.04 ± 0.03
3	0.46 ± 0.01	**11**	2.01 ± 0.04
4	0.59 ± 0.02	**12**	~20
5	0.41 ± 0.02	**13**	0.46 ± 0.05
6	0.43 ± 0.01	**14**	1.23 ± 0.02
7	4.21 ± 0.05	**15**	3.81 ± 0.02
8	>20	**16**	0.75 ± 0.02

[a]The substrate used was cefazolin and inhibitor concentrations were varied between 0.1 and 20 μM.

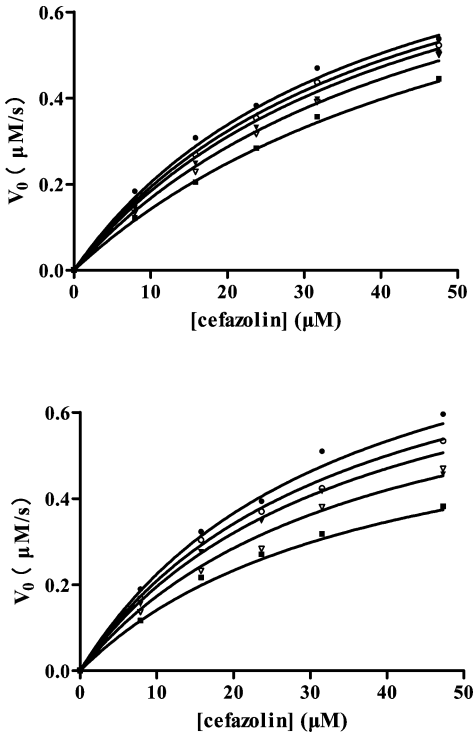


Figure 2. Michaelis−Menten plots of L1-catalyzed hydrolysis of cefazolin in the absence and presence of **4** (top) and **11** (bottom). Concentrations of inhibitor **4**/**11** were 0/0 μM (●), 0.25/2.5 μM (○), 0.5/5.0 μM (▼), 1.0/10 μM (▽), and 2.0/20 μM (■).

for 30 min before adding cefazolin or imipenem, which were then monitored at 262 or 300 nm, respectively, to determine the initial

DOI: 10.1021/acsmedchemlett.7b00058
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

Table 3. MIC Values (μg/mL) of Cefazolin for *E. coli*-DH10B/pBC SK(+)-L1 in the Presence of the N-Substituted Carbamylmethyl Mercaptoacetate Thioethers 1−16 at a Concentration of 1024 μg/mL (A) and 6 and 8 at Concentrations from 4 to 1024 μg/mL (B)[a]

A								
compd	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
MIC	16	8	16	16	16	2	16	2
compd	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
MIC	16	16	16	16	16	16	16	16

B									
compd\conc.	4	8	16	32	64	128	256	512	1024
6	16	16	16	16	16	16	8	8	2
8	16	16	16	16	16	8	8	8	2

[a]In the absence of inhibitor, the MIC of cefazolin for *E. coli*-DH10B/pBC SK(+)-L1 was 16 μg/mL, and for *E. coli* not expressing L1, it was 2 μg/mL.

velocity v_0. We tested different preincubation times (3−60 min) and did not find any difference in residual activity, concluding that these are time-independent inhibitors. Percent inhibition, defined as enzyme activity without inhibitor (100%) minus residual activity with 100 μM inhibitor, is reported for all enzymes tested in Figure S2 in the Supporting Information. The results for IMP-1, ImiS, and L1 as representatives of subclasses B1, B2, and B3, respectively, are summarized in Table 1.

All compounds showed varying degrees of inhibition of the different enzymes. Between IMP-1, ImiS, and L1, compound **9** showed the broadest spectrum of inhibition with more than 70% inhibition. In terms of specific inhibition of a particular enzyme, the compounds were most potent against L1 with compounds **5**, **6**, and **13** reaching the highest percent inhibition with 97%. While we propose that derivatives of this series of thioethers could be developed as more potent broad-spectrum inhibitors or specific inhibitors of the various individual enzymes in future studies, the present series clearly has a preference for L1. Therefore, for the remainder of this report, we will focus on inhibition studies of L1.

The inhibitor concentrations causing 50% decrease of enzyme activity (IC_{50}) of compounds **1**−**16** against L1 were determined in 30 mM Tris, pH 8.5. These kinetic experiments were done in triplicate, and the average values ± standard deviations are reported in Table 2. The kinetic parameters for uninhibited cefazolin hydrolysis by L1 were determined as K_m = 36 ± 2 μM and k_{cat} = 197 ± 1 s^{-1}.

The IC_{50} data indicate that all of the carbamylmethyl mercaptoacetate thioethers inhibit L1 with an IC_{50} value range from 0.4 to 20 μM except for **8** (>20 μM). Compounds **3**, **5**, **6**, and **13** showed the lowest IC_{50} values between 0.41 and 0.46 μM, consistent with their high % inhibition (95−97%) at 100 μM inhibitor concentration. An assessment of the structure−activity relationship (SAR) of these N-substituted carbamylmethyl mercaptoacetate thioethers and their activities reveals some interesting trends. Compounds **1**−**12** all contain an aromatic substituent. Using **1** with a phenyl group (IC_{50} = 0.98 μM) as a reference, **2**−**6** with an electron-donating group on the phenyl ring were more potent inhibitors of L1, with IC_{50} values ranging from 0.41 to 0.59 μM, while **7**−**12** with an electron-accepting group on the phenyl were equally or less potent inhibitors with IC_{50} values ranging from 1.04 to >20 μM. Compounds **5** and **6** with an electron-donating group in para-position of the phenyl were slightly more potent (IC_{50} = 0.41 and 0.43 μM) than **2**−**4** with a substituent in ortho-position of the phenyl (IC_{50} = 0.46−0.59 μM). The electron-accepting nitro and chloro substituents result in more potent inhibitors when in ortho-position (**7** and **10**) than when in meta (**11**) or para (**8** and **12**) position. Among **13**−**16** with aliphatic substituents, **13** with a benzyl substituent was the most potent inhibitor, indicating that an aromatic group is also favorable if not directly linked to the carbamyl nitrogen.

In order to identify the inhibition mode of the carbamylmethyl mercaptoacetate thioethers, we studied inhibition kinetics of L1 with different substrate concentrations and different concentrations of **4** and **11** as representatives of compounds with aromatic substituents with either electron-donating or electron-accepting groups. The concentrations of **4** and **11** were varied from 0 to 2.0 and from 0 to 20 μM, respectively, and substrate (cefazolin) concentrations were varied between 8 and 48 μM. Enzyme and inhibitors were preincubated for 30 min before starting the kinetic assays. The mode of inhibition was determined by generating Michaelis−Menten plots, and K_i values were determined by fitting initial velocity versus substrate concentration at each inhibitor concentration using GraphPad Prism 7. The Michaelis−Menten plots of L1-catalyzed hydrolysis of cefazolin in the absence and presence of compounds **4** and **11** are shown in Figure 2. They indicate that both **4** and **11** exhibit a mixed inhibition mode against L1. The K_i values were determined to be 3.7 ± 0.1 μM for **4** and 31.8 ± 0.3 μM for **11**, respectively (average ± standard deviation of triplicates).

Isothermal titration calorimetry (ITC) has an increasing significance in enzyme kinetic studies owing to its general applicability and sensitivity. In the present work, enzyme-catalyzed progress of hydrolysis of substrate in the absence and presence of inhibitor was monitored on a Malvern MicroCal iTC 200 instrument at 25 °C. The concentration of enzyme was 5 nM, the concentration of substrate (cefazolin) was 160 μM, and the concentration of inhibitor was varied from 0 to 100 μM. The L1-catalyzed hydrolysis progress curves of cefazolin in the absence and presence of **5** at different concentrations are shown in Figure S2.

It is clearly observed that the heat flow decreased gradually with the increase in concentration of **5** from 0 to 100 μM, demonstrating the inhibitory effect of the N-substituted carbamylmethyl mercaptoacetate thioether on thermodynamics. Based on ITC experiments like the one shown in Figure S3 (top) but at different substrate concentrations, reaction rates were calculated and plotted against substrate concentrations for different inhibitor concentrations.[29] The reaction rates from ITC at different substrate concentrations in the presence of 0, 50, and 100 μM **5** are shown in Figure S3 (bottom) and confirm the spectrophotometric results.

The ability of compounds **1**−**16** to restore the antimicrobial activity of cefazolin against bacteria expressing L1 was

DOI: 10.1021/acsmedchemlett.7b00058
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

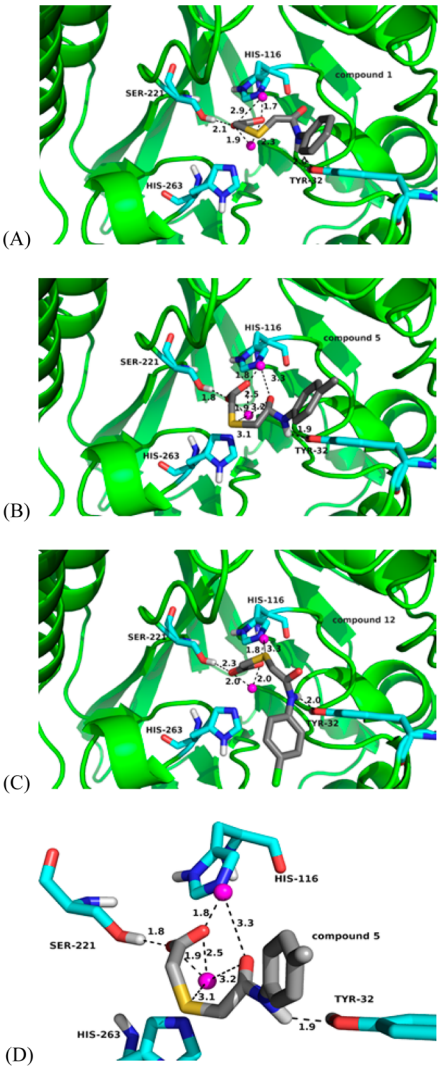


Figure 3. Low-energy docking conformations of compounds **1** (A), **5** (B), and **12** (C) docked into the active site of L1 (PDB code 2AIO[30]). The enzyme backbone is shown as a cartoon in green, and selected residues are shown as sticks colored by element (H, white; C, cyan; N, blue; O, red; S, yellow). Zn(II) ions are shown as magenta spheres; the lower (front) one is Zn2 and the upper (back) Zn1. Compounds **1**, **5**, and **12** are also shown as sticks with the same color code as amino acid residues except C in gray and Cl in green. Characteristic short distances between inhibitors and the protein are indicated by dashed lines. Panel (D) is an enlarged view of the interactions between compound **5** with the L1 active site.

investigated by determining the MICs (see Supporting Information for details) of cefazolin in the presence and absence of 1024 μg/mL **1**−**16** (Table 3A). *E. coli*-DH10B cells harboring pBC SK(+)-L1 were used in these assays. A significant (8-fold) decrease in MIC for **6** and **8** was observed, restoring the antibacterial activity of cefazolin. However, the other compounds did not result in decreased MICs, indicating that there might be problems with entry into the bacteria. Inhibitors alone (1024 μg/mL) without antibiotic did not inhibit cell growth. For the active compounds, **6** and **8**, we also established dose-dependency and found that compound **6** decreased MIC by one serial dilution at 256 μg/mL and compound **8** at 128 μg/mL (Table 3B). No antibacterial effect of the compounds alone at 1024 μg/mL against *E. coli* with and without L1 plasmid was observed.

To explore potential binding modes, compounds **1**, **5**, and **12** as typical representatives of the aromatic N-substituted carbamylmethyl mercaptoacetate thioethers without substituent, with an electron-donating substituent and with an electron-accepting substituent on the phenyl ring, respectively, were docked into the active site of the L1 crystal structure.[30] The lowest-energy docking conformations of the selected clusters of **1**, **5**, and **12** (see Supporting Information for details) are shown in Figure 3A, B, and C, respectively. The carboxylate of all three compounds acts as a bridging ligand of the two Zn(II) ions and forms a hydrogen bond with Ser221, tightly anchoring these inhibitors in the active site, as seen previously with amino acid thioesters.[24,25] The hydrogen of the carbamyl group also forms a hydrogen bond with Tyr32 in all three complexes. The substituent on the phenyl ring seems to affect the orientation of the ring and the adjacent carbamyl group, orienting the carbonyl oxygen toward the two Zn(II) ions in the L1/**5** complex at distances of 3.2 and 3.3 Å (enlarged view in panel D), but not in the other two complexes, providing a rationale for the lower IC_{50} value observed with this compound. With the carbamyl oxygen acting as an additional ligand, **5** is effectively a chelating agent of the two Zn(II) ions, especially Zn2. Such a chelating effect was not observed previously with the amino acid thioesters,[24,25] possibly due to the closer proximity of the thioester oxygen to the carboxylate group in those compounds, not allowing for enough conformational freedom. The addition of a methylene group in the thioethers presented here seems to provide the proper geometry for chelation.

For biomedical applications, the potential toxicity of inhibitors is a major concern. Although no hydrolysis was previously observed *in vitro*, the previously reported thioesters[23−25] can potentially be subject to hydrolysis upon administration in a patient, which would result in mercaptoacetate. Thiol compounds have a high potential to inhibit other zinc enzymes; e.g., L-captopril, a potent MβL inhibitor, also inhibits angiotensin converting enzyme.[31] In fact, while some of the most potent MβL inhibitors reported to date are thiols,[32,33] none of them have been developed into clinically useful drugs. One rationale for designing thioethers as opposed to the previously reported thioesters[24,25] was to avoid the potential hydrolysis resulting in mercaptoacetate and cytotoxicity. Upon incubation in aqueous solution over an extended period of time, especially at lower pH, a representative thioether was clearly more stable than a thioester (Figure S1), suggesting that the thioethers are less prone to hydrolysis. A selection of the newly designed N-substituted carbamylmethyl mercaptoacetate thioethers, **1**, **5**, **7**, and **16**, was subjected to a cytotoxicity assay with mouse fibroblast cells (L929) with different working concentrations (12.5, 25, 50, 100, 200, 400 μM). As shown in Figure S4, none of them affected viability of the L-929 mouse fibroblastic cells at concentrations up to 400 μM, indicating that these thioethers have low cytotoxicity.

In summary, given the enormous clinical importance of metallo-β-lactamases (MβLs), a new scaffold, N-substituted carbamylmethyl mercaptoacetate thioethers, was constructed and characterized by ^{1}H and ^{13}C NMR and MS. The obtained molecules **1**−**16** inhibited a range of MβLs, but preferentially L1, with an IC_{50} value range from 0.4 to >20 μM. Compound **5** was found to be the most potent inhibitor of L1, with an IC_{50} value of 0.41 μM using cefazolin as substrate, with **6**, **3**, and **13** being almost as potent. The mechanism of inhibition of both **4** and **11** was mixed. ITC assays confirmed the findings from UV−vis spectrophotometric experiments. SAR analysis reveals that an

DOI: 10.1021/acsmedchemlett.7b00058
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

electron-donating group on the phenyl ring of aromatic N-substituted carbamylmethyl mercaptoacetate thioethers improves potency of the inhibitors, but an electron-accepting group decreases potency. MIC assays demonstrated that **6** and **8** were able to inhibit L1 expressed by *E. coli* cells and restore the antimicrobial activity of cefazolin to that observed with susceptible cells not expressing L1. The other compounds may not have been able to enter the bacteria and can be optimized in that respect in future studies. Docking studies indicate that the carboxyl group may coordinate the two Zn(II) ions in the active site and hydrogen bond with Ser221 of L1, while the carbamyl group oxygen may act as an additional ligand in the most potent compound **5**, thus rendering the inhibitor a chelating agent, which was not observed with the previously observed thioesters.[24,25] Cytotoxicity tests revealed that **1**, **5**, **7**, and **16** did not affect the viability of mammalian cells at a concentration of up to 400 μM. This was expected due to the decreased likelihood of thioethers to be hydrolyzed, which could result in toxic thiols. These studies demonstrate that the N-substituted carbamylmethyl mercaptoacetate thioethers represent a novel scaffold for the future design of MβL inhibitors.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.7b00058.

Experimental details; NMR and ESI mass data for target compounds; stability data; inhibition data with additional MβLs; ITC data (PDF)

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Author Contributions

K.-W.Y. and P.O. conceived and designed the experiments, analyzed the data, and wrote the paper. Y.-N.C. performed the experiments. Y.-J.Z., Y.X., and C.C. purified proteins. Y.-J.Z. performed cytotoxicity, and W.-M.W. performed ITC assays. All authors have given approval to the final version of the manuscript.

Funding

This work was supported by grants 21272186, 21572179, and 81361138018 (to K.W.Y.) from the National Natural Science Foundation of China.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

ITC, isothermal titration calorimetry; MβL, metallo-β-lactamase; MIC, minimum inhibitory concentration

■ REFERENCES

(1) Berendonk, T. U.; Manaia, C. M.; Merlin, C.; Fatta-Kassinos, D.; Cytryn, E.; Walsh, F.; Burgmann, H.; Sorum, H.; Norstrom, M.; Pons, M. N.; Kreuzinger, N.; Huovinen, P.; Stefani, S.; Schwartz, T.; Kisand, V.; Baquero, F.; Martinez, J. L. Tackling antibiotic resistance: the environmental framework. *Nat. Rev. Microbiol.* **2015**, *13*, 310−7.

(2) Anonymous. The antibiotic alarm. *Nature* **2013**, *495*, 141.

(3) King, D. T.; Strynadka, N. C. J. Targeting metallo-β-lactamase enzymes in antibiotic resistance. *Future Med. Chem.* **2013**, *5*, 1243−63.

(4) Bush, K. Proliferation and significance of clinically relevant β-lactamases. *Ann. N. Y. Acad. Sci.* **2013**, *1277*, 84−90.

(5) Ambler, R. P. The structure of β-lactamases. *Philos. Trans. R. Soc., B* **1980**, *289*, 321−31.

(6) Drawz, S. M.; Bonomo, R. A. Three decades of β-lactamase inhibitors. *Clin. Microbiol. Rev.* **2010**, *23*, 160−201.

(7) Garau, G.; García-Sáez, I.; Bebrone, C.; Anne, C.; Mercuri, P.; Galleni, M.; Frère, J.-M.; Dideberg, O. Update of the standard numbering scheme for class B β-lactamases. *Antimicrob. Agents Chemother.* **2004**, *48*, 2347−9.

(8) Felici, A.; Amicosante, G.; Oratore, A.; Strom, R.; Ledent, P.; Joris, B.; Fanuel, L.; Frere, J.-M. An overview of the kinetic parameters of class B β-lactamases. *Biochem. J.* **1993**, *291*, 151−5.

(9) Lassaux, P.; Hamel, M.; Gulea, M.; Delbruck, H.; Mercuri, P. S.; Horsfall, L.; Dehareng, D.; Kupper, M.; Frere, J.-M.; Hoffmann, K.; Galleni, M.; Bebrone, C. Mercaptophosphonate compounds as broad-spectrum inhibitors of the metallo-β-lactamases. *J. Med. Chem.* **2010**, *53*, 4862−76.

(10) Bebrone, C.; Lassaux, P.; Vercheval, L.; Sohier, J. S.; Jehaes, A.; Sauvage, E.; Galleni, M. Current challenges in antimicrobial chemotherapy: focus on β-lactamase inhibition. *Drugs* **2010**, *70*, 651−79.

(11) Ehmann, D. E.; Jahić, H.; Ross, P. L.; Gu, R. F.; Hu, J.; Durand-Réville, T. F.; Lahiri, S.; Thresher, J.; Livchak, S.; Gao, N.; Palmer, T.; Walkup, G. K.; Fisher, S. L. Kinetics of avibactam inhibition against class A, C, and D β-lactamases. *J. Biol. Chem.* **2013**, *288*, 27960−71.

(12) McGeary, R. P.; Schenk, G.; Guddat, L. W. The applications of binuclear metallohydrolases in medicine: recent advances in the design and development of novel drug leads for purple acid phosphatases, metallo-β-lactamases and arginases. *Eur. J. Med. Chem.* **2014**, *76*, 132−44.

(13) Walter, M. W.; Felici, A.; Galleni, M.; Soto, R. P.; Adlington, R. M.; Baldwin, J. E.; Frere, J.-M.; Gololobov, M.; Schofield, C. J. Trifluoromethyl alcohol and ketone inhibitors of metallo-β-lactamases. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2455−8.

(14) Toney, J. H.; Hammond, G. G.; Fitzgerald, P. M.; Sharma, N.; Balkovec, J. M.; Rouen, G. P.; Olson, S. H.; Hammond, M. L.; Greenlee, M. L.; Gao, Y. D. Succinic acids as potent inhibitors of plasmid-borne IMP-1 metallo-β-lactamase. *J. Biol. Chem.* **2001**, *276*, 31913−8.

(15) Hiraiwa, Y.; Saito, J.; Watanabe, T.; Yamada, M.; Morinaka, A.; Fukushima, T.; Kudo, T. 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.

(16) Mohamed, M. S.; Hussein, W. M.; McGeary, R. P.; Vella, P.; Schenk, G.; Abd El-hameed, R. H. 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.

(17) Simm, A. M.; Loveridge, E. J.; Crosby, J.; Avison, M. B.; Walsh, T. R.; Bennett, P. M. Bulgecin A: a novel inhibitor of binuclear metallo-β-lactamases. *Biochem. J.* **2005**, *387*, 585−90.

(18) Walter, M. W.; Valladares, M. H.; Adlington, R. M.; Amicosante, G.; Baldwin, J. E.; Frere, J.-M.; Galleni, M.; Rossolini, G. M.; Schofield, C. J. Hydroxamate inhibitors of *Aeromonashydrophila* AE036 metallo-β-lactamase. *Bioorg. Chem.* **1999**, *27*, 35−40.

(19) Toney, J. H.; Fitzgerald, P. M. D.; Grover-Sharma, N.; Olson, S. H.; May, W. J.; Sundelof, J. G.; Vanderwall, D. E.; Cleary, K. A.; Grant, S. K.; Wu, J. K.; Kozarich, J. W.; Pompliano, D. L.; Hammond, G. G. Antibiotic sensitization using biphenyl tetrazoles as potent inhibitors of *Bacteroides fragilis* metallo-β-lactamase. *Chem. Biol.* **1998**, *5*, 185−96.

(20) Siemann, S.; Evanoff, D. P.; Marrone, L.; Clarke, A. J.; Viswanatha, T.; Dmitrienko, G. I. N-arylsulfonyl hydrazones as inhibitors of IMP-1 metallo-β-lactamase. *Antimicrob. Agents Chemother.* **2002**, *46*, 2450−7.

(21) Cornaglia, G.; Giamarellou, H.; Rossolini, G. M. Metallo-β-lactamases: a last frontier for β-lactams? *Lancet Infect. Dis.* **2011**, *11*, 381−93.

(22) Li, J.; Nation, R. L.; Turnidge, J. D.; Milne, R. W.; Coulthard, K.; Rayner, C. R.; Paterson, D. L. Colistin: the re-emerging antibiotic for

DOI: 10.1021/acsmedchemlett.7b00058
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

multidrug-resistant gram-negative bacterial infections. *Lancet Infect. Dis.* **2006**, *6*, 589−601.
(23) Payne, D. J.; Bateson, J. H.; Gasson, B. C.; Proctor, D.; Khushi, T.; Farmer, T. H.; Tolson, D. A.; Bell, D.; Skett, P. W.; Marshall, A. C.; Reid, R.; Ghosez, L.; Combret, Y.; Marchand-Brynaert, J. Inhibition of metallo-β-lactamases by a series of mercaptoacetic acid thiol ester derivatives. *Antimicrob. Agents Chemother.* **1997**, *41*, 135−40.
(24) Liu, X. L.; Shi, Y.; Kang, J. S.; Oelschlaeger, P.; Yang, K. W. Amino acid thioester derivatives: a highly promising scaffold for the development of metallo-β-lactamase L1 inhibitors. *ACS Med. Chem. Lett.* **2015**, *6*, 660−4.
(25) Liu, X. L.; Yang, K. W.; Zhang, Y. J.; Ge, Y.; Xiang, Y.; Chang, Y. N.; Oelschlaeger, P. Optimization of amino acid thioesters as inhibitors of metallo-β-lactamase L1. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 4698−701.
(26) Zhang, Y. L.; Yang, K. W.; Zhou, Y. J.; LaCuran, A. E.; Oelschlaeger, P.; Crowder, M. W. Diaryl-substituted azolylthioacetamides: inhibitor discovery of New Delhi metallo-β-lactamase-1 (NDM-1). *ChemMedChem* **2014**, *9*, 2445−8.
(27) Yang, S. K.; Kang, J. S.; Oelschlaeger, P.; Yang, K. W. Azolylthioacetamide: a highly promising scaffold for the development of metallo-β-lactamase inhibitors. *ACS Med. Chem. Lett.* **2015**, *6*, 455−60.
(28) Crowder, M. W.; Walsh, T. R.; Banovic, L.; Pettit, M.; Spencer, J. Overexpression, purification, and characterization of the cloned metallo-beta-lactamase L1 from Stenotrophomonas maltophilia. *Antimicrob. Agents Chemother.* **1998**, *42*, 921−6.
(29) Poduch, E.; Bello, A. M.; Tang, S. S.; Fujihashi, M.; Pai, E. F.; Kotra, L. P. Design of inhibitors of orotidine monophosphate decarboxylase using bioisosteric replacement and determination of inhibition kinetics. *J. Med. Chem.* **2006**, *49*, 4937−45.
(30) Spencer, J.; Read, J.; Sessions, R. B.; Howell, S.; Blackburn, G. M.; Gamblin, S. J. Antibiotic recognition by binuclear metallo-β-lactamases revealed by X-ray crystallography. *J. Am. Chem. Soc.* **2005**, *127*, 14439−44.
(31) King, D. T.; Worrall, L. J.; Gruninger, R.; Strynadka, N. C. J. New Delhi metallo-β-lactamase: structural insights into β-lactam recognition and inhibition. *J. Am. Chem. Soc.* **2012**, *134*, 11362−5.
(32) Oelschlaeger, P.; Ai, N.; DuPrez, K. T.; Welsh, W. J.; Toney, J. H. Evolving carbapenemases: can medicinal chemists advance one step ahead of the coming storm? *J. Med. Chem.* **2010**, *53*, 3013−27.
(33) King, D. T.; Strynadka, N. C. J. Targeting metallo-β-lactamase enzymes in antibiotic resistance. *Future Med. Chem.* **2013**, *5*, 1243−63.

DOI: 10.1021/acsmedchemlett.7b00058
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX