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Graphical Abstract

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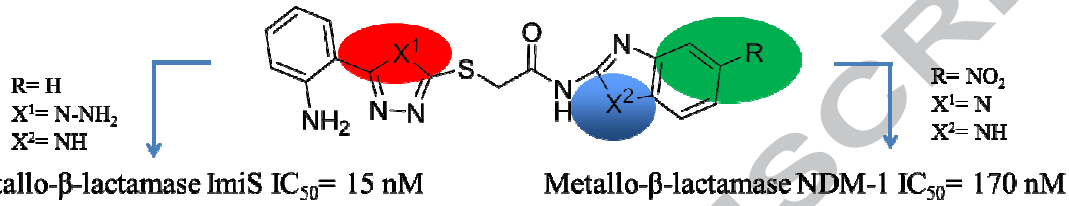

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ABSTRACT

In an effort to develop new inhibitors of metallo-β-lactamases (MβLs), twenty-eight azolylthioacetamides were synthesized and assayed against MβLs. The obtained benzimidazolyl and benzioxazolyl substituted **1**-**19** specifically inhibited the enzyme ImiS, and **10** was found to be the most potent inhibitor of ImiS with an IC_{50} value of 15 nM. The nitrobenzimidazolyl substituted **20**-**28** specifically inhibited NDM-1, with **27** being the most potent inhibitor with an IC_{50} value of 170 nM. Further studies with **10**, **11**, and **27** revealed a mixed inhibition mode with competitive and uncompetitive inhibition constants in a similar range as the IC_{50} values. These inhibitors resulted in a 2-4-fold decrease in imipenem MIC values using *E. coli* cells producing ImiS or NDM-1. While the source of uncompetitive (possibly allosteric) inhibition remains unclear, docking studies indicate that **10** and **11** may interact orthosterically with Zn2 in the active site of CphA, while **27** could bridge the two Zn(II) ions in the active site of NDM-1 *via* its nitro group.

Following the discovery of the antibiotic penicillin in the 1920s,[1] β-lactam antibiotics were developed in the 1940s as miracle drugs to treat bacterial infections.[2] β-Lactams exert their antibacterial effect by inhibiting transpeptidases (penicillin binding proteins) that are responsible for the biosynthesis of the bacterial cell wall consisting of peptidoglycan.[3] However, the overuse of antibiotics has resulted in a large number of bacteria that are resistant to these drugs. The most important mechanism of β-lactam resistance is the production of β-lactamases. β-Lactamases render bacteria resistant to many commonly used antibiotics, including penicillins, cephalosporins and carbapenems, by catalyzing the hydrolysis of the C-N bond of the β-lactam ring of the drugs, and thus inactivating the antibiotics.[3,4]

To date, there have been more than 2,000 distinct β-lactamases identified, and these enzymes have been categorized into classes A–D, based on amino acid sequence homology.[5] Enzymes of classes A, C, and D, also known as serine β-lactamases (SβLs), use an active-site serine to perform a nucleophilic attack on the β-lactam carbonyl, ultimately leading to a cleaved β-lactam ring. Class B enzymes, also known as metallo-β-lactamases (MβLs), utilize either 1 or 2 equivalents of Zn(II) for full catalytic activity to hydrolyze β-lactam antibiotics.[6,7] The MβLs are further divided into subclasses B1-B3, based on amino acid sequence homology and Zn(II) content.[7]

The New Delhi metallo-β-lactamase-1 (NDM-1), a B1 subclass MβL, was initially discovered in *Klebsiella pneumoniae* in 2008,[8] and has since rapidly spread globally.[9, 10] The plasmid-encoded NDM-1 gene has been shown to horizontally transfer to other pathogenic bacteria. ImiS, a B2 subclass enzyme, has a narrow substrate profile including carbapenems, which have been called "last resort"antibiotics.[11, 12] The MβLs including NDM-1 and ImiS hydrolyze all clinical carbapenems and have grown into a serious threat to the health of human beings.[9] There are no known clinical inhibitors of the MβLs to date.[13]

Given the enormous biomedical importance of MβLs, there has been a large amount of effort in identifying novel inhibitors. King *et al.* reported that the aspergillomarasmine A is a potent inhibitor of NDM-1 and VIM-2.[14] Biphenyl tetrazoles,[15]

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succinic[16] and amino acid-derived thiols[17] also displayed inhibitoryactivity on IMP-1. Chiou *et al.* reported that Ebselen inhibits NDM-1 by forming a S-Se bond with the Cys221 residue in the active site of the enzyme.[18] Also, some broad-spectrum inhibitors of the MβLs have been reported, including mercaptophosphonates, thiomandelic acid, thiols,[19] and mercaptothioenolates.[20]

Recently, we found that the azolylthioacetamides provide a highly promising scaffold for the development of MβL inhibitors.[21-23] Specifically, the aromatic carboxyl substituted azolylthioacetamides inhibit ImiS,[22] while the triazolylthioacetamides inhibit NDM-1.[23] In addition, some of the azolylthioacetamides exhibit broad-spectrum inhibitory activity against CcrA, NDM-1, ImiS, and L1, representing members of all three subclasses.[21] Our goal is to develop inhibitors of MβLs and to use these inhibitors in combination with the β-lactam antibiotics to combat infections caused by the MβL-producing bacteria. To further explore the structure-activity relationships of compounds with the azolylthioacetamide scaffold, twenty-eight new compounds were synthesized and characterized. Their potential as MβL inhibitors was evaluated with the enzymes NDM-1, ImiS and L1, which are representatives of the B1, B2 and B3 subclasses of MβLs, respectively. Also, the ability of these inhibitors to restore the potency of existing antibiotics against antibiotic resistant strains was evaluated. Furthermore, molecular docking was employed in studying possible interactions between the inhibitors and their preferred MβLs.

Twenty-eight new azolylthioacetamides (Fig. 1) were synthesized as shown in Scheme S1 in the Supplementary Data. Firstly, the mercaptoazoles were synthesized by a previously reported method.[21] *o*-Diaminobenzene reacted with NH_2-CN or Br-CN to give amino-benzimidazoles.[24] The intermediates amino-benzimidazole and amino-benzoxazole were acylated with chloroacetyl chloride to offer α-chloroacetamides.[21] Finally, under alkaline conditions, the azoles and α-chloroacetamides were cross-linked by nucleophilic substitution to afford the target products azolylthioacetamides (**1**-**11** and **13**-**28**).[21-23] Bifunctional **12** was synthesized by a different route. All compounds were characterized by ^{1}H and ^{13}C NMR and confirmed by MS.

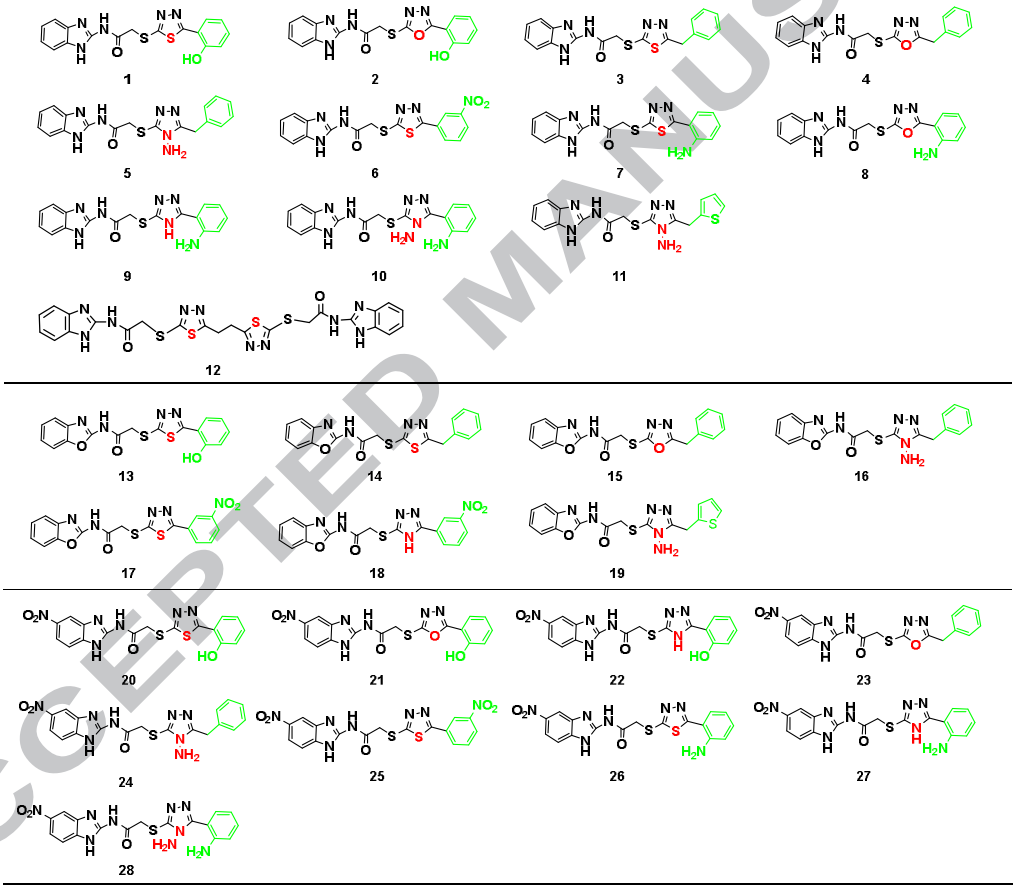

Fig. 1. Structures of the synthesized azolylthioacetamides. The varied atoms and substituents are shown in red and green, respectively.

To test whether these azolylthioacetamides were inhibitors of MβLs, enzymes from the three subclasses B1 (NDM-1), B2 (ImiS) and B3 (L1) were over-expressed and purified as previously described.[25-28] The inhibition experiments were conducted as described in the Supplemental Data.

The inhibition studies indicated that, while none of the azolylthioacetamides tested had any activity against L1 at concentrations up to 500 μM (data not shown), they had specific inhibitory activity against either NDM-1 or ImiS. The inhibitor concentrations causing 50% decrease of enzyme activity (IC_{50}) are listed in Table 1. **1**-**19** specifically inhibited ImiS, with **1**-**12** with benzimidazole (Fig. 1) exhibiting a lower IC_{50} value range of 0.015-18 μM than **13**-**19** with benzoxazole (IC_{50} =29-384 μM). **5**, **10**, **11**, **16** and **19** with an amino-triazole showed lower IC_{50} values than the corresponding compounds with the triazole, oxazole or thiazole group. **10** gave the lowest IC_{50} value of 15 nM against ImiS. On the other hand, **20**-**28** with a nitrobenzimidazole significantly inhibited NDM-1 with an IC_{50} value range of 17 nM

to 6.4 μM. This observation suggests that the nitro group of these inhibitors may be involved in binding selectively to the Zn (II) ion(s) in the dinuclear active site of the enzyme. **27** exhibited the lowest IC_{50} value of 170 nM against NDM-1, which is similar to the data (150 nM) of the best NDM-1 inhibitor among the triazolylthioacetamides that we recently reported.[23]

Table 1. IC_{50} values of azolylthioacetamides against metallo-β-lactamases NDM-1 and ImiS

Compds	IC_{50} (μM)		Compds	IC_{50} (μM)	
	NDM-1	ImiS		NDM-1	ImiS
1	-	0.33±0.02	**15**	-	384±7
2	-	0.63±0.06	**16**	-	29±1
3	-	18±3	**17**	-	133±3
4	-	3.0±0.1	**18**	-	378±9
5	-	0.27±0.04	**19**	-	53±1
6	-	1.8±0.1	**20**	6.4±0.2	-
7	-	1.2±0.2	**21**	1.3±0.1	-
8	-	1.7±0.1	**22**	5.9±0.4	-
9	-	0.94±0.07	**23**	0.50±0.09	-
10	-	0.015±0.009	**24**	0.73±0.09	-
11	-	0.077±0.01	**25**	5.1±0.2	-
12	-	0.39±0.07	**26**	2.5±0.3	-
13	-	196±4	**27**	0.17± 0.03	-
14	-	259±8	**28**	1.1±0.1	-

The substrate for NDM-1 and ImiS was imipenem; -: no inhibition at an inhibitor concentration of 500 μM.

To identify the inhibition mode of the azolylthioacetamides against MβLs, we studied inhibition kinetics of ImiS and NDM-1 with different substrate concentrations and different concentrations of **10** and **11** as representatives of compounds with a benzimidazole group and **27** as a representative with a nitro substituted benzimidazole group. The concentrations of **10** were varied from 0 to 125 nM and those of **11** and **27** from 0 to 250 nM. Imipenem concentrations were varied from 0 to 150 nM. Enzyme and inhibitors were pre-incubated for 5 min before starting the kinetic assays. The mode of inhibition was determined by generating Lineweaver–Burk plots, and K_i values were obtained by fitting initial velocity *versus* substrate concentration at each inhibitor concentration using SigmaPlot 12.0.

The Lineweaver–Burk plots of ImiS- and NDM-1-catalyzed hydrolysis of imipenem in the absence and presence of **10**, **11** and **27** are shown in Fig. 2. The results indicate that all three compounds exhibit a competitive and uncompetitive mixed inhibition mode. The K_{ic}/K_{iu} values were determined to be 22 ± 4/11 ± 2 nM (alpha = 0.52) for **10**, 110 ± 50 /80 ± 30 nM (alpha = 0.72) for **11**, and 550 ± 90/310 ± 50 nM (alpha = 0.56) for **27** (average ± standard deviation of triplicates). The K_i values of **27** are similar to the data of the best azolylthioacetamide inhibitor against NDM-1 (490 nM),[23] while the K_i values of **10** against ImiS are significantly (~50-fold) smaller than the data of the best compounds (1.2 μM) that we recently reported,[22] indicating that the activity of this azolylthioacetamide is greatly improved. We can currently not identify the source of the apparent uncompetitive inhibition mode; it might be due to binding of the compounds to allosteric sites of the enzymes. We hypothesize that the competitive inhibition mode is due to orthosteric inhibition, i.e., binding of the compounds to the MβLs' active sites.

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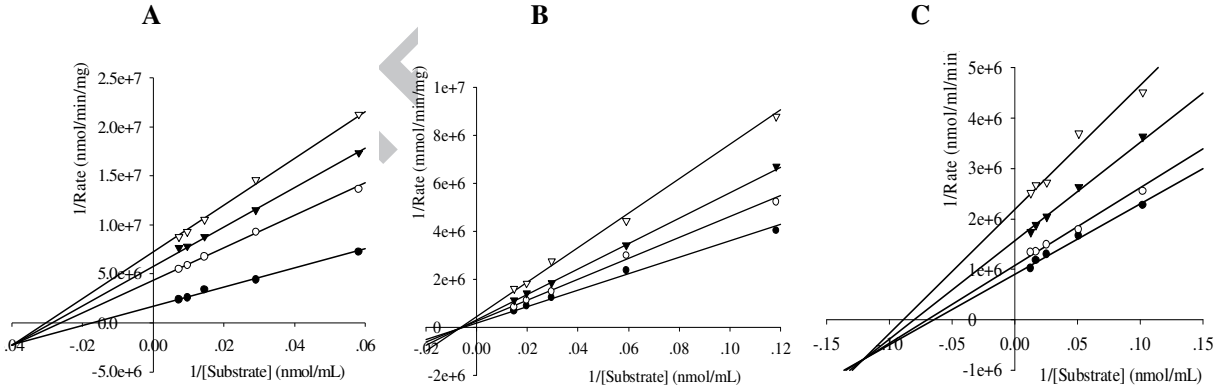

Fig. 2. Lineweaver–Burk plots of ImiS- and NDM-1-catalyzed hydrolysis of imipenem in the absence and presence of **10** (**A**), **11** (**B**), and **27** (**C**). Concentrations of inhibitors **10/11** and **27** were 0/0 (●), 31.25/62.5 (○), 62.5/125 (▼) and 125/250 nM (▽).

The ability of the azolylthioacetamides to restore the antimicrobial activity of imipenem against bacteria expressing ImiS or NDM-1 was investigated by determining the minimum inhibitory concentrations (MICs) of the antibiotic in the presence and absence of 16 μg/mL **1**-**28**. The results are listed in Table 2. *E. coli* BL21 (DE3) harboring pET26b-NDM-1 or pET26b-ImiS were used to evaluate these inhibitors. A significant (2-4-fold) decrease in MIC of imipenem was observed for **1**-**19** with *E. coli* cells expressing ImiS, while **20**-**28** showed no effect. Conversely, **20**-**28** decreased imipenem MICs with *E. coli* cells expressing NDM-1, while **1**-**19** had no effect. The ability of these azolylthioacetamides to partially restore the antibacterial activity of the antibiotic is consistent with their inhibitory effect on the MβLs. No antibacterial effect of the compounds alone against *E. coli* with and without ImiS or NDM-1 plasmid was observed.

Table 2. Antibacterial activities (MICs, μg/mL) of imipenem against *E. coli* BL21 (DE3) with ImiS or NDM-1 in the absence and presence of inhibitors **1**-**28** at a concentration of 16 μg/mL. MIC of imipenem for *E. coli* cells that don't expressing MβL was 0.125 μg/mL.

E. coli-NDM-1				***E. coli***-ImiS			
MIC (μg/mL) (control)	16			1			
Compds	MIC (μg/mL)	Compds	MIC (μg/mL)	Compds	MIC (μg/mL)	Compds	MIC (μg/mL)
1	16	**15**	32	**1**	0.5	**15**	0.5
2	16	**16**	16	**2**	0.5	**16**	0.5
3	16	**17**	16	**3**	0.5	**17**	0.5
4	16	**18**	16	**4**	0.25	**18**	0.25
5	16	**19**	16	**5**	0.5	**19**	0.5
6	16	**20**	8	**6**	0.5	**20**	1
7	32	**21**	8	**7**	0.5	**21**	1
8	16	**22**	8	**8**	0.25	**22**	1
9	16	**23**	4	**9**	0.5	**23**	1
10	16	**24**	4	**10**	0.25	**24**	1
11	16	**25**	8	**11**	0.25	**25**	1
12	16	**26**	8	**12**	0.25	**26**	2
13	16	**27**	4	**13**	0.5	**27**	1
14	16	**28**	8	**14**	0.5	**28**	1

To explore potential orthosteric binding modes, three typical representatives of azolylthioacetamides were docked into the active sites of their preferred targets: **10** and **11** into CphA, (in lieu of ImiS, which has not been crystallized, yet, and with which it shares 96% sequence identity), and **27** into NDM-1. The conformations shown in Fig. 3 are the lowest-energy conformations of those clusters, with binding energies of -6.1, -5.9, and -10.3 kcal/mol for the CphA/**10**, CphA/**11** and NDM-1/**27** complexes, respectively. While the CphA/ImiS inhibitors are more potent in experiment, the docking binding energy is lower with the NDM-1 inhibitors, which is most likely due to a second Zn(II) ion in NDM-1 resulting in stronger electrostatic interactions in the model.

In terms of possible binding mode these docking calculations indicate that in compounds **10** and **11**, which exhibited the lowest IC_{50} and K_i values with ImiS, the N-amino triazole may interact face-on with Zn2 with N---Zn(II) distances between 3.0 and 4.1 Å (Fig. 3, panels **B** and **C**). Interaction with active site Zn(II) ions is typically the key force for inhibitor binding to MβLs, as observed for instance with biphenyl tetrazoles,[15] succinic acids,[16] or thiol compounds.[17] The triazoles also form hydrogen bonds with His118 and His196 of CphA. In addition, both compounds interact with Asn233 and Thr157 via the amide group. Compound **10** has additional interactions with Zn2 and Lys224 via its aryl amino group (Fig. **3B**). This finding is consistent with the slightly lower IC_{50} and K_i values obtained from experiment, relative to **11**. Contrary to the previous findings,[22] the triazole ring of compound **27** did not show any interactions with Zn1 or Zn2 of NDM-1 in the docked conformations (Fig. **3E**). Instead, the nitro group acted as a bridging ligand of Zn1 and Zn2, which is reminiscent of the binding mode of a micromolar inhibitor of IMP enzymes recently studied.[29] The nitro group as a zinc ligand has been demonstrated previously in a crystal structure of Zn-dependent carboxypeptidase A in complex with 2-benzyl-3-nitropropanoic acid.[30] Overall, **27** indicates several possible interactions with the NDM-1 active site, including the Zn(II) ions, Lys211, Gln123 and Asn220, that are comparable to the binding mode of hydrolyzed ampicillin from a crystal structure (Fig. **3D**, PDB code 4HL2). While other conformations were obtained from the docking study as well, the majority of the conformations showed interactions similar to those highlighted in the selected conformations, namely interactions of the N-amino triazole of **10** and **11** with Zn2 of CphA and the nitro group of **27** with Zn1 and Zn2 of NDM-1.

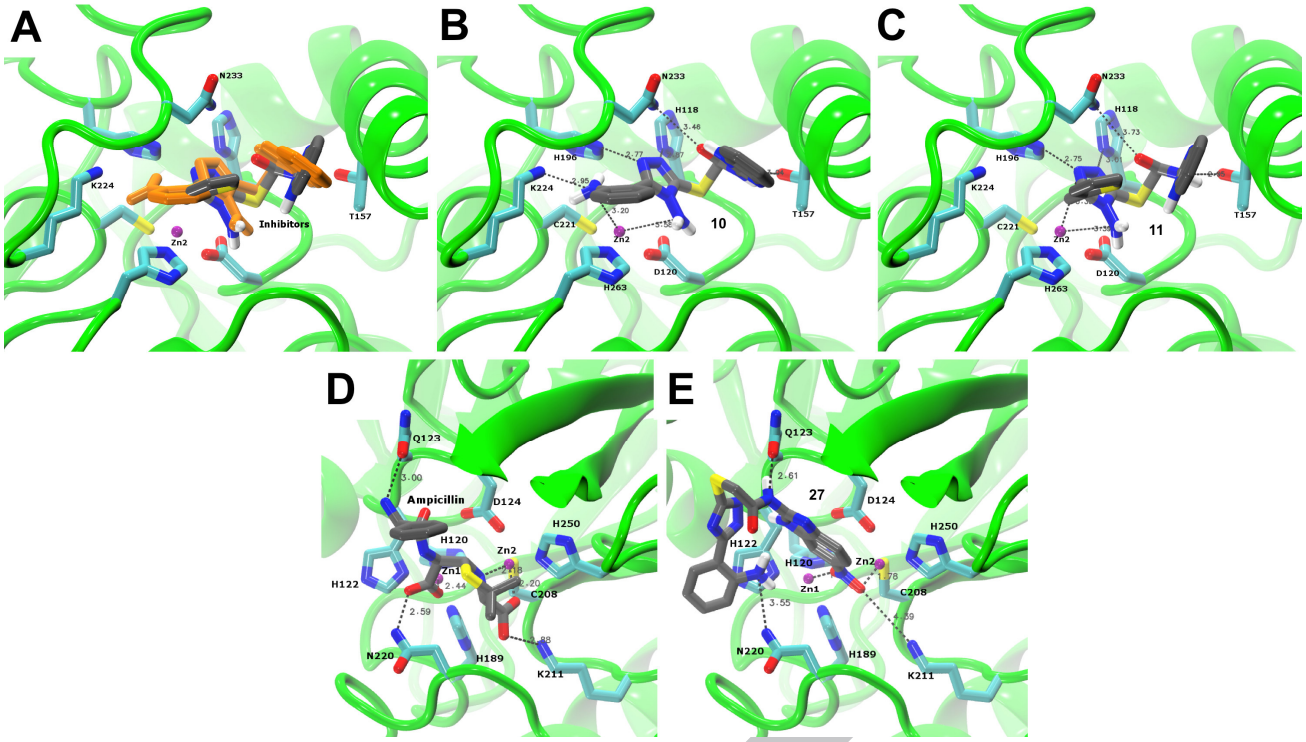

Fig. 3. Lowest-energy conformations of **10** and **11** docked into the active site of CphA (PDB code 2QDS) and **27** docked into the active site of NDM-1 (PDB code 4HL2). Both enzymes are depicted as follows: backbone as cartoon in green and side chains of selected residues as licorice colored by atom (H, white; C, cyan; N, blue; O, red; S, yellow). The Zn(II) ions are shown as purple spheres. **A** shows superimposition of compounds **10** and **11** in the CphA active site. **10** is depicted as licorice colored by atom (same colors as for protein residues except C in gray), and **11** as orange sticks. **B**, **C**, **D**, and **E** are detailed views of **10**, **11**, hydrolyzed ampicillin and **27**, respectively, displaying key enzyme residues and indicating interactions between the inhibitors and enzyme residues with dashed lines. All images were generated with VMD.[31]

In summary, twenty-eight new azolylthioacetamides were synthesized, characterized by NMR and confirmed by MS. Biological activity evaluation indicates that the azolylthioacetamides with benzimidazolyl and benzoxazolyl **1**-**19** specifically inhibit ImiS, with the lowest IC_{50} value of 15 nM. However, the azolylthioacetamides with nitrobenzimidazolyl **20**-**28** significantly inhibit NDM-1, with the lowest IC_{50} value of 170 nM. These inhibitors, in combination with imipenem, resulted in a 2-4-fold decrease in imipenem MIC values with *E. coli* cells expressing ImiS or NDM-1. Docking studies suggest that the N-amino triazole of **10** and **11** interacts with Zn2 in the active site of CphA, while, inhibitor **27** uses its nitro group to bridge the two Zn(II) ions in the active site of NDM-1. The information gained in this work is valuable for the further development of MβL inhibitors.

Acknowledgments

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References and notes

1. Fleming, A. *Infect. Dis.* **1980**, 2, 129.
2. Abraham, E. P.; Chain, E.; Fletcher, C.M.; Florey H. W.; Gardner A. D.; Heatley N. G.; Jennings M. A. *Eur. J. Clin. Pharmacol.* **1992**, 42, 3.
3. Fisher, J. F.; Meroueh, S.; Mobashery, O. S. *Chem. Rev*. **2005**, 105, 395.
4. King, D. T. S.; Sobhanifar, N. C.; Strynadka, N. C. J. *Protein Sci.* **2016**, 25, 787.
5. Ambler, R. P.; Trans, P. R. Soc. Lond, B. *Biol. Sci.* **1980**, 289, 321.
6. Crowder, M. W.; Spencer, J.; Vila, A. J. *Acc. Chem. Res*. **2006**, 39, 721.
7. Bebrone, C. *Biochem. Pharmacol.* **2007**, 74, 1686.
8. Yong, D. M. Toleman, A. Giske, C. G.; Cho H. S.; Sundman K.; Lee K.; Walsh T. R. *Antimicrob. Agents Chemother.* **2009**, 53, 5046
9. Walsh, T. R. *Int. J. Antimicrob. Agents*. **2010**, 36 (Suppl 3), S8.
10. Mojica, M. F.; Bonomo, R. A.; Fast, W. *Curr. Drug Targets.* **2016**, 17, 1029.
11. Schneider, K. D.; Karpen, M. E.; Bonomo, R. A.; Leonard, D. A.; Powers, R. A. *Biochem.* **2009**, 48, 11840.
12. Papp-Wallace, K. M.; Endimiani, A.; Taracila, M. A.; Bonomo, R. A. *Antimicrob. Agents Chemother.* **2011**, 55, 4943.
13. Cornaglia, G.; Giamarellou, H.; Rossolini, G. M. *Lancet Infect. Dis*. **2011**, 11, 381.
14. King, A. M.; Reid-Yu, S. A.; Wang, W. L.; King, D. T.; De Pascale, G.; Strynadka, N. C.; Walsh, T. R.; Coombes, B. K.; Wright, G. D. *Nature* **2014**, 510, 503.
15. Toney, J. H.; Fitzgerald, P. M.; 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. *Chem. Biol.* **1998**, 5, 185;
16. 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. *J. Biol. Chem.* **2001**, 276, 31913;
17. Arjomandi, O. K.; Husseina, W. M.; Vella, P.; Yusof, Y.; Sidjabat, H. E.; Schenk, G.; McGeary, R. P. *Eur. J. Med. Chem.* **2016**, 114, 318.
18. Chiou, J. C.; Wan, S. B.; Chan, K. F.; So, P. K.; He, D.; Chan, E. W.; Chan, T. H.; Wong, K. Y.; Tao, J.; Chen, S. *Chem. Commun.* **2015**, 51, 9543.
19. Lienard, B. M.; Horsfall, L. E.; Galleni, M.; Frere, J. M.; Schofield, C. J. *Bioorg. Med. Chem. Lett*. **2007**, 17, 964.

20. Brem, J. S.; van Berkel, S. S.; V. Aik, W.; Rydzik, A.; M.; Avison, M. B.; Pettinati, I.; Umland, K. D.; Kawamura, A.; Spencer, J.; Claridge, T.D.; McDonough, M. A.; Schofield, C. *Nat. Chem.* **2014**, 6, 1085.
21. Zhang, Y. L.; Yang, K. W.; Zhou, Y. J.; LaCuran, A. E.; Oelschlaeger, P.; Crowder, M. W. *Chem. Med. Chem.* **2014**, 9, 2445.
22. Yang, S. K..; Kang, J. S.; Oelschlaeger, P.; Yang, K. W. *ACS Med. Chem. Lett*. **2015**, 6, 455.
23. Zhai, L.; Zhang, Y. L.; Kang, J. S.; Oelschlaeger, P.; Xiao, L.; Nie, S. S.; Yang, K.. W. *ACS Med. Chem. Lett.* **2016**, 7, 413.
24. Mobinikhaledi, A.; Kalhor Hatami, M. Synthesis of some new benzimidazolyl thioxoacetamide derivatives. Asian. J. Chem. 2010, 16,
25. Yang, H.; Aitha, M.; Hetrick, A. M.; Richmond, T. K.; Tierney, D. L.; Crowder, M. W. *Biochem.* **2012**, 51, 3839.
26. Wang, Z.; Benkovic, S. J. *J. Biol. Chem.* **1998**, 273, 22402-22408;
27. Crawford, P. A.; Sharma, N.; Chandrasekar, S.; Sigdel, T.; Walsh, T. R.; Spencer, J.; Crowder, M. W. *Protein Expr. Purif.* **2004**, 36, 272-279;
28. Crowder, M. W.; Walsh, T. R.; Banovic, L.; Pettit, M.; Spencer, J. *Antimicrob. Agents Chemother.* **1998**, 42, 921.
29. LaCuran, A. E.; Pegg, K. M.; Liu, E. M.; Bethel, C. R.; Ai, N.; Welsh, W. J.; Bonomo, R. A.; Oelschlaeger, P. *Antimicrob. Agents Chemother.* **2015**, 59, 7299.
30. Wang, S. H.; Wang, S. F.; Xuan, W.; Zeng, Z. H.; Jin, J. Y.; Mad, J.; Tian, G. R. *Bioorg. Med. Chem.* **2008**, 16, 3596.
31. Humphrey, W.; Dalke, A.; Schulten, K. *J. Molec. Graphics.* **2009**, 14, 33.

Supplementary Data

Supplementary material that may be helpful in the review process should be prepared and provided as a separate electronic file. That file can then be transformed into PDF format and submitted along with the manuscript and graphic files to the appropriate editorial office.