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BRIEF COMMUNICATION

Azolylthioacetamides as potential inhibitors of New Delhi metallo-β-lactamase-1 (NDM-1)

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Abstract

Thirteen azolylthioacetamides was synthesized and characterized. Biological activity assays with MβLs revealed that all of these compounds (except **3** and **6**) gained exhibited inhibitory activity on NDM-1, with an IC_{50} value ranging from 3.8 to 26.4 μM. Inhibitors **11** and **13**, in combination with cefazolin, resulted in a four-fold decrease in MIC of the antibiotic against *Escherichia coli* cells expressing NDM-1. Docking studies revealed that the inhibitors **7**, **9**, and **13** bound to active site of NDM-1.

β-Lactam antibiotics are relatively inexpensive but effective antimicrobial agents [1, 2]. To withstand the action of β-lactam antibiotics, bacteria produce β-lactamases, which hydrolyze the C–N bond of the β-lactam ring, this represents the most common mechanism of resistance [3]. There have been more than 2000 distinct β-lactamases identified [4], and these enzymes have been classified into A–D four distinct classes based on molecular properties [5]. Class A, C, and D enzymes contain a serine residue as nucleophile agent in the reaction, which are generically termed as serine β-lactamases (SβLs). Class B β-lactamases, also known as metallo-β-lactamases (MβLs), are characterized by the presence of one or two Zn(II) ions that are essential for their function and by a larger substrate diversity [6]. MβLs have been further divided into subclasses B1 to B3 [7]. Inhibition of β-lactamases presents a promising strategy to prevent the hydrolysis of β-lactam antibiotics. For some SβLs, this strategy has been proven successful and SβL inhibitors are already in clinical use [8]. However, so far there are no clinically approved drugs targeting MβLs.

New Delhi metallo-β-lactamase (NDM-1) poses a global threat to human health due to its ability to hydrolyze nearly all β-lactam antibiotics and its rapid worldwide spread. NDM-1 belongs to the B1 subclass MβL that requires a dinuclear Zn(II) center to catalyze hydrolysis of a variety of substrates [9]. Additionally, NDM-1 gene is borne on a readily transferable plasmid, which facilitates its transmission [10].

Facing the emergence of drug resistance mediated by MβLs, a large number of MβL inhibitors have been reported, such as Aspergillomarasmine A (AMA) [11], dipicolinic acids [12], rhodanine [13], cyclic boronates [14], ebselen [15], etc. Recently, we found that the azolylthioacetamides are a highly promising scaffold for the development of MβL inhibitors [16–20]. Specifically, the aromatic carboxyl substituted azolylthioacetamides inhibit ImiS [16], while the triazolylthioacetamides inhibit NDM-1. In addition, some of the azolylthioacetamides exhibit broad-spectrum inhibitory activity against CcrA, NDM-1, ImiS, and L1, as representatives of three subclasses MβLs [18]. 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 relationship of the azolylthioacetamides, in this work, the novel azolylthioacetamides were synthesized and evaluated with MβLs.

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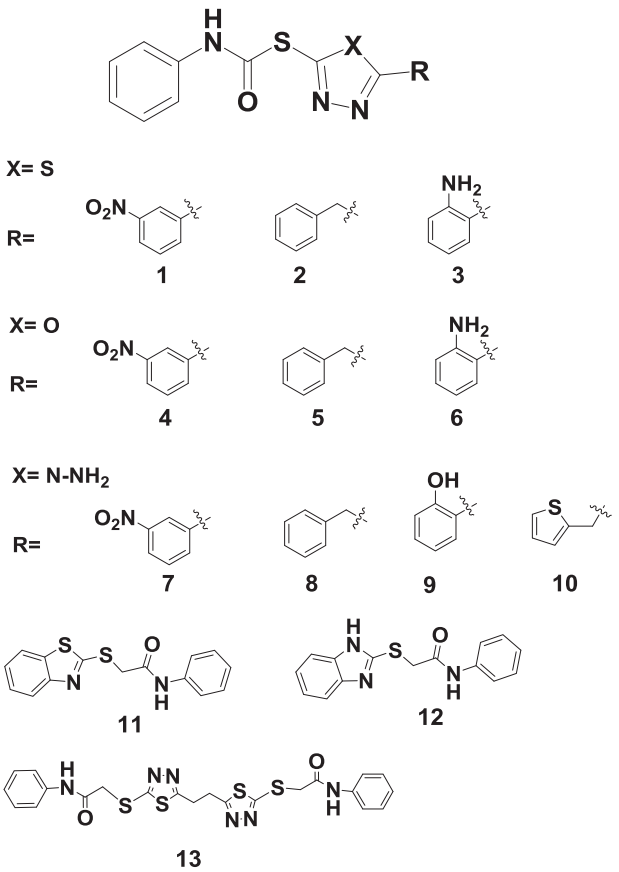

Fig. 1 Structures of the synthesized azolylthioacetamides

Thirteen azolylthioacetamides (Fig. 1) were synthesized as the synthesized procedure and routes (Scheme S1) in supporting information. The MβLs from subclasses B1 (NDM-1), B2 (ImiS), and B3 (L1) were overexpressed and purified as previously described (Shown in supporting information). To test whether these azolylthioacetamides were inhibitors of the MβLs, the percent inhibition of all compounds were tested against MβLs on an Agilent UV8453 spectrometer using cefazolin as substrate of NDM-1 and L1, and imipenem as substrate of ImiS. The substrate concentration was 60 μM and inhibitor concentration was 20 μM. Enzymes and inhibitor were pre-incubated for 5 min before starting the kinetic test. The percentage inhibition of most tested compounds against L1 and ImiS was low, but high percent inhibition on NDM-1 was observed (Fig. S1).

Concentrations causing 50% decrease of enzyme activity (IC_{50}) by these compounds were determined. The substrate concentration was 60 μM, and inhibitor concentration was varied between 2.5 and 50 μM. The IC_{50} values of the tested compounds are listed in Table 1. It can be observed that all inhibitors, except **3** and **6** (poor solubility), inhibited NDM-1 with IC_{50} value range of 3.80–26.4 μM. The IC_{50} values of **1** and **4** are slightly smaller than those of **2** and **5**, respectively, revealing that 2-nitrophenyl group as substitute results in stronger inhibitory activity against NDM-1 than the benzyl group. The nitro group has been demonstrated to be the coordinating group of Zn(II) ion in the crystal structure of carboxypeptidase A in complex with 2-benzyl-3-nitropropanoic acid (PDB code: 2RFH) [21]. Compounds **11** and **12** exhibited an IC_{50} value of 6.0 and 23.4 μM, respectively, indicating that the benzothiazole moiety results in higher inhibitory activity than the benzimidazole. Furthermore, among these azolylthioacetamides, **13** showed the lowest IC_{50} value of 3.80 μM against NDM-1, suggesting that the molecules with bifunctional groups, i.e., bi-azolylthioacetamides, could be a valuable scaffold for development of the more potent MβL inhibitors.

The ability of azolylthioacetamides to restore antimicrobial activity of cefazolin against bacteria expressing NDM-1 was investigated by determining the minimum inhibitory concentrations (MICs) of the antibiotic in the absence and presence of **1–13** [22]. *E. coli* BL21(DE3) harboring pET26b-NDM-1 was used to evaluate these inhibitors. The results (listed in Table 1) showed that only compounds **11** and **13** resulted in a significant (four-fold) decrease in MIC of cefazolin against *E. coli* cells expressing NDM-1 at a concentration up to 128 μg ml^{-1}. 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 NDM-1 plasmid was observed.

Also, **7**, **9**, and **13** as the representatives of inhibitors were subjected to a cytotoxicity assay using mouse fibroblasts cells (L-929) with different working concentrations (12.5, 25, 50, 100, 200, 400 μM). As shown in Fig. S2, more than 90% of the cells tested maintained viability in the presence of the inhibitors at concentration up to 400 μM, indicating that these azolylthioacetamides have low cytotoxicity.

To explore potential orthosteric binding modes, compounds **7**, **9**, and **13** were docked into the active sites of NDM-1. The conformations shown in Fig. S3 are the lowest-energy conformations of those clusters. The docking calculations show that **7** and **9** form tight interactions with the active site of NDM-1, with the nitro group and hydroxyl group, respectively, bridging to Zn1 and Zn2 site of NDM-1, the triazole interacting with the backbone amide groups of Gln123 and Asp124, the amide carbonyl interacting with the backbone amide groups of Gln123, and the amide nitrogen interacting with the Glu152 side chain. In addition, the 2-phenolyl hydroxyl group in compound **9** hydrogen bonds with the Asp124 side chain. All interactions described between inhibitor **7** or **9** and NDM-1 were at distances of less than 2.9 Å. The most potent inhibitor of NDM-1, compound **13**, fits tightly into the active site of NDM-1. The

Table 1 IC_{50} and MIC values of azolylthioacetamides on NDM-1 and *E. coli* expressing NDM-1, respectively

Inhibitors	IC_{50}[a] (μM)	MIC[b,c] (μg ml^{-1})	Inhibitors	IC_{50}[a] (μM)	MIC[b, c] (μg ml^{-1})
1	10.0 ± 0.8	32	**8**	19.1 ± 0.5	64
2	12.5 ± 0.7	32	**9**	13.5 ± 0.7	32
3	>50	–	**10**	16.1 ± 1.1	32
4	18.3 ± 0.9	32	**11**	6.0 ± 0.7	16
5	26.4 ± 0.6	64	**12**	23.4 ± 0.9	32
6	>50	–	**13**	3.8 ± 0.4	16
7	18.3 ± 1.0	32			

– The compound is separated out

[a]The substrate used was cefazolin

[b]MICs of cefazolin against *E. coli* with NDM-1 in the absence and presence of inhibitors **1–13** at a concentration of 128 μg ml^{-1}

[c]MICs of cefazolin for *E. coli* cells that express or don't express MβL was 64 and 2 μg ml^{-1}, respectively

binding mode of **13**, which is structurally significantly different from **7** and **9**, is different: one of the amide carbonyls bridges the Zn(II) ions, while the nitrogen of the same amide interacts with the Asn220 side chain and the adjacent thiadiazole interacts with the Gln123 backbone amide; the other amide nitrogen interacts with the side chain of Met154 and the backbone of Val55.

In summary, thirteen azolylthioacetamides were synthesized and characterized. Biochemical evaluation revealed that the azolylthioacetamides (except **3** and **6**) gained inhibited NDM-1, exhibiting an IC_{50} value ranging from 3.8 to 26.4 μM. The compounds **11** and **13**, in combination with cefazolin, resulted in a four-fold decrease in MIC of the antibiotic against *E. coli* cells expressing NDM-1 at a concentration up to 128 μg ml^{-1}. The cytotoxicity assays indicated that azolylthioacetamides **7**, **9**, and **13** had low cytotoxicity at a concentration up to 400 μM. Docking studies revealed that **7** and **9** form stable interactions in the active site of NDM-1, bridging the Zn(II) ions via the nitro or hydroxyl group, respectively, while other moieties interact with the backbones of Gln123 and Asp124, and the side chains of Asn220 and Glu152. The binding mode of **13** is different from that of **7** and **9**, which coordinates to the Zn(II) ions via one of its amide carbonyls and interact with the Asn220 and Met154 side chains and the Gln123 and Val55 backbones. The information gained in this work is valuable for further development of MβLs inhibitors.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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