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NOTA analogue: A first dithiocarbamate inhibitor of metallo-β-lactamases

En Zhang [a,c,*], Ming-Ming Wang [a], Shu-Chao Huang [b], Shuai-Min Xu [a], De-Yun Cui [a], Yuan-Li Bo [a], Peng-Yan Bai [a], Yong-Gang Hua [a], Chun-Ling Xiao [b,*], Shangshang Qin [a,c,*]

[a] School of Pharmaceutical Sciences, Institute of Drug Discovery and Development, Key Laboratory of Advanced Pharmaceutical Technology, Ministry of Education of China, Zhengzhou University, Zhengzhou 450001, PR China
[b] Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, PR China
[c] Collaborative Innovation Center of New Drug Research and Safety Evaluation, Henan Province, Zhengzhou 450001, PR China

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ABSTRACT

The emergence of antibiotic drug (like carbapenem) resistance is being a global crisis. Among those resistance factors of the β-lactam antibiotics, the metallo-β-lactamases (MBLs) is one of the most important reasons. In this paper, a series of cyclic dithiocarbamate compounds were synthesized and their inhibition activities against MBLs were initially tested combined with meropenem (MEM) by in vitro antibacterial efficacy tests. Sodium 1,4,7-triazonane-1,4,7-tris(carboxylodithioate) (compound **5**) was identified as the most active molecule to restore the activity of MEM. Further anti-bacterial effectiveness assessment, compound **5** restored the activity of MEM against *Escherichia coli*, *Citrobacter freundii*, *Proteus mirabilis* and *Klebsiella pneumonia*, which carried resistance genes of bla_{NDM-1}. The compound **5** was non-hemolytic, even at a concentration of 1000 μg/mL. This compound was low toxic toward mammalian cells, which was confirmed by fluorescence microscopy image and the inhibition rate of HeLa cells. The *Ki* value of compounds **5** against NDM-1 MBL was 5.63 ± 1.27 μM. Zinc ion sensitivity experiments showed that the inhibitory effect of compound **5** as a MBLs inhibitor was influenced by zinc ion. The results of the bactericidal kinetics displayed that compound **5** as an adjuvant assisted MEM to kill all bacteria. These data validated that this NOTA dithiocarbamate analogue is a good inhibitor of MBLs.

Antibiotics paved the way which is an unprecedented medical and social development, and is indispensable in all health systems.[1] The reason for antibiotic resistance is complicated, and it contains a variety of complex factors.[2,3]

β-Lactam antibiotics are among the most widely used classes of antibiotics that have been used for the treatment of infectious diseases for many years.[4] The emergence of β-lactamases has been an ongoing problem since the clinical introduction of β-lactams 60 years ago. β-Lactamases can make β-lactams ineffective by hydrolyzing the β-lactam ring, which is the key structure for β-lactam activity. The overuse of β-lactams in the clinical setting as well as for animal production has exacerbated this problem.[5] Extended spectrum β-lactamases have emerged, which can inactivate many new generation penicillins and cephalosporins but not carbapenems.[6] The carbapenem class of β-lactam antibiotics (such as imipenem and MEM) were considered agents of last resort because of their resistance to the action of β-lactamases.[7] However, the consumption of carbapenem has been steadily growing over the past two decades as a consequence of extended spectrum β-lactamases. Predictably, enzymes capable of hydrolyzing carbapenem antibiotics have emerged and spread predominantly in Gram-negative bacteria worldwide, leading to acquired resistance to this class of drugs.[8–10]

Carbapenem-hydrolyzing enzymes (also known as carbapenemases) are categorized into classes A, B, C and D, depending on their amino acid sequence homologies.[11] Explicit reviews on the structure, mechanism, genetic diversity, epidemiology and clinical management of β-lactamases have been reported.[12,13] Classes A, C and D comprise serine-β-lactamases that covalently bind to a hydrolyzed β-lactam *via* a nucleophilic serine moiety. Most of the serine-β-lactamases are inhibited by compounds like clavulanic acid, sulbactam, and tazobactam. Metallo-β-lactamases (MBLs, Ambler class B) contain one or two zinc ions in their active site that promote nucleophilic attack of the β-lactams *via* a polarized water

* Corresponding authors at: School of Pharmaceutical Sciences, Institute of Drug Discovery and Development, Key Laboratory of Advanced Pharmaceutical Technology, Ministry of Education of China, Zhengzhou University, Zhengzhou 450001, PR China (E. Zhang, S. Qin).
E-mail addresses: zhangen@zzu.edu.cn (E. Zhang), qinshangshang@126.com (S. Qin).

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molecule. MBLs are not susceptible to any of the serine-β-lactamase inhibitors and can hydrolyze most β-lactam antibiotics applied in the clinical.[14–16]

To date, although no clinically useful MBLs inhibitors has been approved, there have been many types of MBLs inhibitors with good activity against MBLs, such as thiol-based captopril[17,18], dicarboxylic acid,[19,20] and so on.[15] Among these inhibitors, the number of inhibitors which can restore antibiotic sensitivity to CRE, occupied a small part.[21–23] Aspergillomarasmine A (AMA, Fig. 1) is a naturally occurring fungal product identified in 2014 that is a rapid and potent inhibitor of NDM-1 and VIM-2 MBLs. AMA can fully restore the activity of MEM against bacteria producing NDM-1 and VIM-2 in vitro and in vivo.[24] Furthermore, metal-chelating agents 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA, Fig. 1) and its analogue 1,4,7,10-tetraazacyclod-odecane-1,4,7,10-tetraacetic acid (DOTA)[25–27] can also be used as a potent inhibitors of NDM, IMP and VIM-type MBLs.[28] The activity of MEM against bacteria producing MBLs was restored by combining with either NOTA or DOTA. Some approved drugs containing thiols (such as L-Captopril, Fig. 1) were reported as inhibitors of NDM-1, VIM-1, and IMP-7.[29] In 2015, spiroindoline-thiadiazole SIT-Z5 (Fig. 1) were developed as a new kind of efficient MBLs inhibitor in vitro and in vivo.[30]

Although, examples of chelating agents used clinically are limited and are generally used for in vivo metal overdose which is caused by disease conditions, such as heavy metal toxicity.[31] Recently, more chelating agents have been studied for use in unconventional applications, such as the development of selective chelating agents for the treatment of cancer[32] and neurodegenerative diseases.[33]

During the course of our investigation on new structures with good biologic activity, we recently disclosed that several dithiocarbametes had good anticancer activity.[34,35] Inspired by these exciting results of new MBLs inhibitors (Fig. 1)[24,28,30] and extensive biologic activity of dithiocarbamates,[36] we present preliminary studies of NOTA dithiocarbamates as MBLs inhibitors.

The synthetic route towards target compounds **1**–**8** is shown in Schemes 1–3. Compound **1** was synthesized from piperazine and

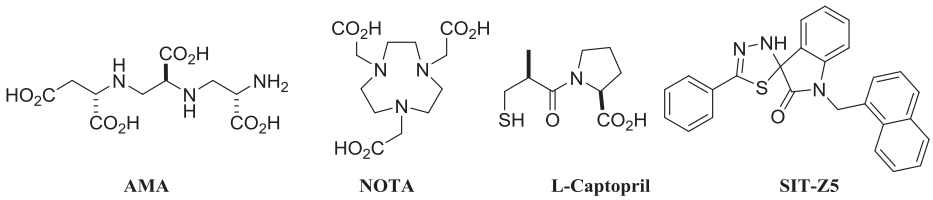
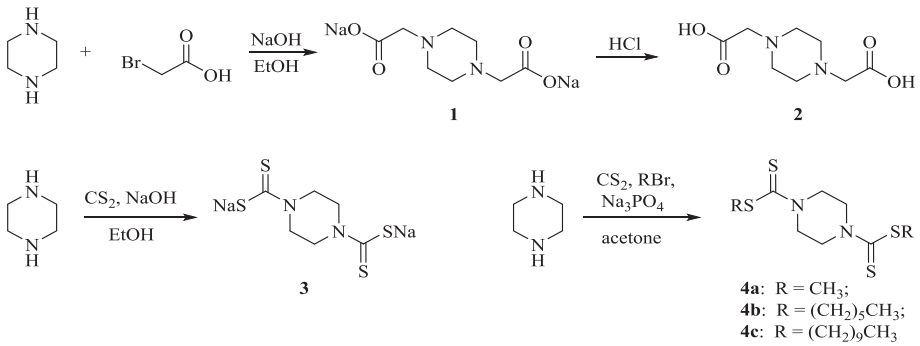
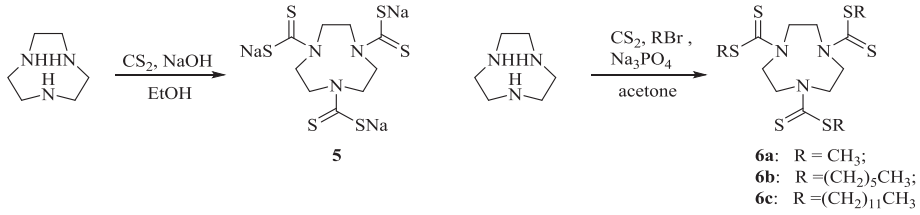


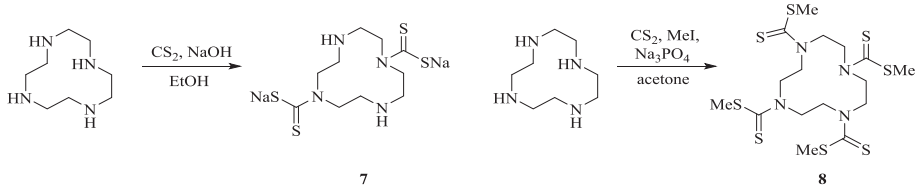
Fig. 1. Structures of some good MBL inhibitors.



Scheme 1. Syntheses of piperazine derivatives.



Scheme 2. Syntheses of NOTA derivatives.



Scheme 3. Syntheses of DOTA derivative.