(–)-Epicatechin gallate serves as a novel new delhi metallo-β-lactamase-1 (NDM-1) inhibitor

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Abstract: The β-lactam antibiotic resistance caused by NDM-1 has become a major crisis of global public health. We have previously screened out (–)-epicatechin gallate (ECG) as a potent NDM-1 inhibitor. We further discussed its inhibitory effect and action mode in the present study. According to our results, ECG reversibly inactivated NDM-1 in a non-competitive mode, with an IC_{50} value of 4.48 μM. ECG effectively recovered the activity of several β-lactam antibiotics against resistant strain harboring *blaNDM-1*. Especially, the effects on carbapenems were worth mentioning. The zinc supplement assay indicated a zinc-related mechanism of ECG. Different from traditional chelating agents, it showed low toxicity both *in vivo* and *in vitro*. In a word, our findings provided a promising NDM-1 inhibitor, ECG, which was able to assist carbapenems against NDM-1-producing strain.
Keywords: NDM-1; (–)-Epicatechin gallate; Inhibitor; Antibiotics resistance

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1. Introduction

Bacterial resistance mainly attributed to the widespread use and the abuse of antibiotics represents a global health threat[1]. Lack of available drugs, resistant pathogens limit the choice of clinical treatment[2]. As estimated, there are over 700 thousand people who died of bacterial resistance annually worldwide, despite tremendous efforts, this number continues to grow[1]. Once considered as the last line of defense against Gram-negative bacteria infection, carbapenem antibiotics suffer resistance as well[3]. In the priority pathogen list published by the World Health Organization (WHO) in 2017, three carbapenem-resistant Gram-negative bacteria have been pointed out in "critical" category[4].

The production of carbapenemases is a vital mechanism of carbapenem resistance[5]. Carbapenemases are distributed in Ambler classes A, B, and D, and among them, classes A and D belong to serine β-lactamases (SBLs), while class B belongs to metal β-lactamases (MBLs)[5]. In the past few years, the approval and combination application of β-lactam and SBL inhibitors have effectively alleviated the antibiotic resistance induced by SBLs[6]. Contrarily, the resolution of β-lactam resistance caused by MBLs is urgent[6].

Distinguished by the structure of the mental center, MBLs are divided into three subclasses: B1, B2, and B3[7]. B1 class is the most clinically important, of which NDM-1 gets lots of attention for its strong hydrolysis ability[7]. NDM-1 was first reported in 2008, characterized by an αβ/βα sandwich fold and a binuclear zinc center[7,8]. Rapidly propagating among bacteria, NDM-1 can hydrolyze all β-lactam antibiotics except for monobactams[8]. Since it was discovered, scholars from all over the world have devoted themselves to searching for effective

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inhibitors of NDM-1, and a series of active substances that exert an inhibitory effect on NDM-1 by targeting zinc ions and/or amino acid residues in catalytic pockets have been obtained. Regrettably, apart from boron-based inhibitor taniborbactam in phase 3 clinical studies (with cefepime), there is no clinically available NDM-1 inhibitor[9].

The natural product is thought to be a big reservoir for medicinal compounds. (–)-Epicatechin gallate (ECG), the third major catechin extracted from green tea, possesses a series of pharmacological functions, such as anti-oxidation, anticancer, anti-inflammatory, anti-apoptosis, anti-obesity, and neuroprotection[10–15]. We have previously constructed a high-throughput screening model for NDM-1 and found ECG as a potent NDM-1 inhibitor[16]. To the best of our knowledge, it has not been reported on inhibition of β-lactamases before. Therefore, in this article, we aimed to reveal the inhibitory efficacy and the probable action mechanism of ECG on NDM-1 to acquire a novel inhibitor of NDM-1.

2. Method

2.1. Materials

ECG was purchased from J&K Scientific Company. Piperacillin was obtained from Shanghai Macklin Biochemical Co., Ltd. Other antibiotics (cefoperazone, cephalothin, meropenem, and biapenem) were provided by the National Institutes for Food and Drug Control. EDTA was supplied by Sangon Biotech (Shanghai) Co., Ltd. $ZnCl_2$ was purchased from J&K Scientific Company.

Engineered expression strain BL21 (DE3)/pET30a (+)-NDM-1 harboring *blaNDM-1* gene was provided by Professor Xuefu You of the Institute of Medicinal Biotechnology, Chinese Academy of medical sciences. The Vero cell line and HepG2 cell line were presented by Professor Yuhuan Li and Qiyang He, respectively.

2.2. IC_{50} assay

NDM-1 was incubated with a gradient concentration of ECG in 10 mM HEPES (pH 7.5) at 37 ºC for 15 min. After that, meropenem was added to the enzymatic assay system with a final concentration of 50 μM. The absorbance of meropenem at 300 nm was determined at 37 ºC. The IC_{50} value was analyzed using GraphPad Prism 5.

2.3. Inhibition mode assays

Reversibility assay was carried out by incubation of gradient NDM-1 with different concentrations of ECG. Subsequently, the hydrolysis of 50 μM meropenem was recorded under each combination. The V-[E] plot was drawn with the concentration of NDM-1 as the X-axis and the initial rate as the Y-axis.

The inhibition mode was detected by Lineweaver-Burk plot. Briefly, after incubation of NDM-1 with a gradient concentration of ECG at 37 ºC for 15 min, gradient meropenem was added to initiate the reaction. The curve was drawn with the reverse of substrate's concentration as the X-axis and the reverse of the initial rate as the Y-axis.

2.4. Fluorescence quenching experiment

The fluorescence quenching experiment was carried out in a 200-μL system in 96-well black plate. The concentration of NDM-1 was approximately 647 μg/mL, the ECG was respectively added at 0, 3.125, 6.25, 12.5, 25 and 50 μg/mL. Then the fluorescence spectrum of NDM-1 was measured with the excitation wavelength of 270 nm, and the emission wavelength ranging from 290 to 500 nm.

2.5. Surface plasmon resonance experiment

The surface plasmon resonance experiment was performed using Reichert 2SPR. Briefly, the dextran chip surface was activated by the mixture of EDC and NHS. Then the NDM-1 injected was bound to the dextran surface based on the amine coupling method. When the fixed amount of NDM-1 reached the appropriate level, the chip was subsequently blocked by ethanolamine. We injected gradient ECG dissolved in PBST containing 1.25% DMSO through the NDM-1 surface, and PBST (1.25% DMSO) was used as blank control and set every three samples. The spectrum was monitored and shown in real-time, and the binding constant was analyzed by Trace Drawer software.

2.6. *In vitro* susceptibility tests

In vitro susceptibility test was determined in a 200-μL system in 96-well plate with about 2×10^5 CFU/mL inoculation of BL21 (DE3)/pET30a(+)-NDM-1. The antibiotics selected were diluted by the two-fold dilution method, from 1024 to 0.5 μg/mL for piperacillin, from 256 to 0.125 μg/mL for cefoperazone and cephalothin, and from 64 to 0.03125 μg/mL for meropenem and biapenem. Briefly, 32 μg/mL ECG was then added to each antibiotic to observe combined effects. The plates were sealed with sealing film and covered with tin foil. After cultured at 37 ºC for about 20 h, the plates were observed and recorded.

Microdilution checkerboard analysis was carried out as described above, with the difference that the checkerboards were set up with 11 concentrations (0.625 to 64 μg/mL) of each antibiotic and four concentrations (16 to 128 μg/mL) of ECG.

2.7. Zinc supplement assay

The testing system and method were the same as above, and the final concentration was 16 μg/mL for both meropenem and ECG. We added an equimolar amount of $ZnCl_2$ to the system to see whether the role of ECG was changed. Additionally, we did the same with EDTA as a positive control. After cultured at 37 ºC for about 30 h, the plates were observed and recorded.

2.8. Toxicity assessment

The Vero cells and HepG2 cells were seeded into a 96-well plate with the inoculation of 2×10^4 and 5×10^4 /mL, respectively, followed by incubation at 37 ºC for 24 h. The ECG was diluted and added to the cell with the final concentration ranging from 3.125 to 200 μg/mL. After incubation for 48 h, the cell viability was detected by CCK8.

The acute toxicity study was performed on healthy mice (SPF) weighing 16–18 g. There were six mice (three males and three females) in the experimental group. The mice were orally administered with 500 mg/kg ECG and continuously observed for 1 week, and the survival and abnormal signs were recorded every day.

3. Results

3.1. ECG inhibits NDM-1 activity

The structure of ECG was shown in Figure 1A, and the chemical formula was $C_{22}H_{18}O_{10}$. The further biological assay revealed that ECG inhibited NDM-1 in a dose-dependent manner, with an IC_{50} value of 4.48 μM when meropenem was the substrate (Fig. 1B). To elucidate the types of inhibition, the V-[E] plot and Lineweaver-Burk plot were utilized. As the results suggested, ECG inhibited NDM-1 *via* a reversible and noncompetitive mechanism (Fig. 1C–D).

3.2. The interaction between ECG and NDM-1

To explore whether there was an interaction between ECG and NDM-1, the fluorescence quenching experiment and surface plasmon resonance experiment were performed. According to the fluorescence quenching experiment (Fig. 2A), the addition of ECG caused a dose-dependent drop in fluorescence emission intensity. The result revealed that ECG indeed interacted with NDM-1, changing the microenvironment of aromatic amide acids inside NDM-1, and consequently altering the emission spectra. Consistent with this deduction, the SPR experiment also proved the medium strength interaction between ECG and NDM-1, with a K_D value of $8.02e^{-5}$ M (Fig. 2B).

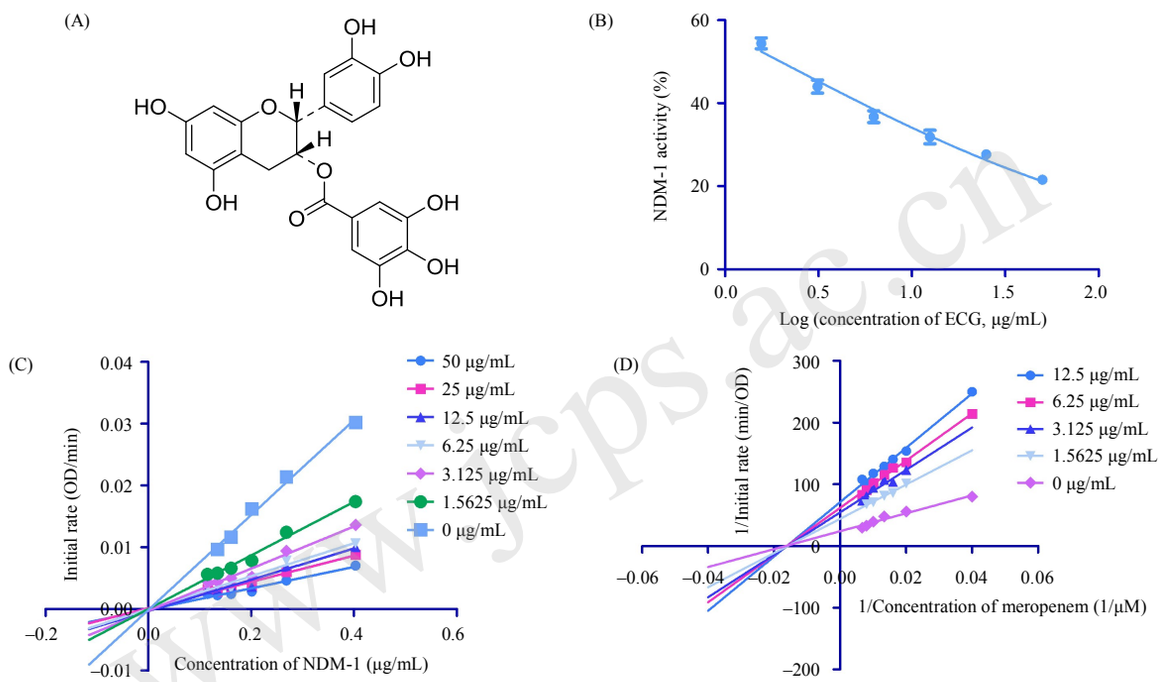

Figure 1. ECG inhibits NDM-1 in a dose-dependent manner. (A) The chemical structure of ECG; (B) Determination of the IC_{50} value of ECG against NDM-1; (C) Reversibility assay; (D) Lineweaver-Burk plot to examine the inhibition model of ECG against NDM-1.

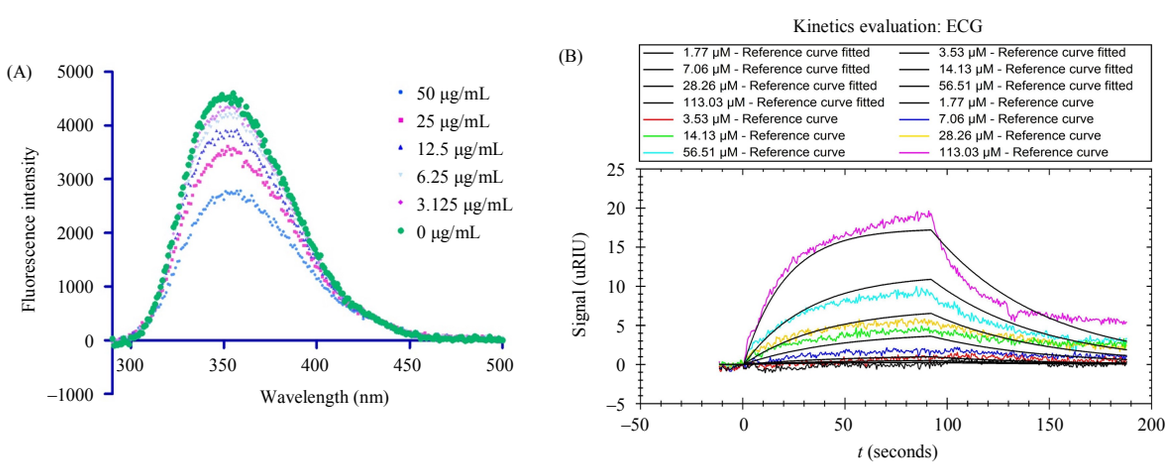

Figure 2. ECG interacts with NDM-1 of medium strength. (A) The fluorescence emission spectrum of NDM-1 with ECG; (B) SPR spectrum after adding ECG with gradient concentration.

3.3. ECG restores β-lactam antibiotic efficacy against NDM-1-producing strain

As shown in Table 1, the strain harboring *blaNDM-1* gene was highly resistant to the tested antibiotics, including penicillins (piperacillin), cephalosporins (cefoperazone and cephalothin), and carbapenems (meropenem and biapenem). To test the synergistic effects of ECG on β-lactam antibiotics, ECG was added to different antibiotics respectively at 32 μg/mL, in likewise, EDTA was added as a positive control. According to the results, the addition of 32 μg/mL ECG reduced the minimal inhibitory concentration (MIC) of piperacillin, cefoperazone, meropenem, and biapenem to 2-fold, 4-fold, 4-fold, and 4-fold, respectively.

Given that the synergism of ECG to carbapenems was remarkable, we further detected the antibacterial activity of carbapenems in the combination of gradient concentration of ECG. As expected, ECG dose-dependently lowered the MIC of meropenem and biapenem. Meanwhile, ECG alone didn't exhibit antibacterial activity within the range of 16–128 μg/mL (Fig. 3). When treated with 128 μg/mL ECG, the MIC of meropenem and biapenem against NDM-1-producing strain was reduced to 0.5 and 0.25 μg/mL, respectively.

3.4. ECG inactivates NDM-1 *via* a zinc-related mechanism

Considering that interaction with zinc ion inside the active pocket of NDM-1 was thought to be the dominant and effective strategy in inhibitor research and development, we attempted to explore whether ECG worked in this way as well. As shown in Figure 4,

Table 1. MICs of different β-lactams in combination with ECG against NDM-1-producing strain.

Antibiotics	*E. coli* BL21(DE3)/pET30a(+) MIC (μg/mL)	*E. coli* BL21(DE3)/pET30a(+)-*blaNDM-1* MIC (μg/mL)		
		0	ECG 32 μg/mL	EDTA 32 μg/mL
Piperacillin	< 0.5	128	64	< 0.5
Cefoperazone	< 0.125	128	32	< 0.125
Cephalothin	1	256	256	1
Meropenem	< 0.03125	64	16	< 0.03125
Biapenem	< 0.03125	2	0.5	0.0625

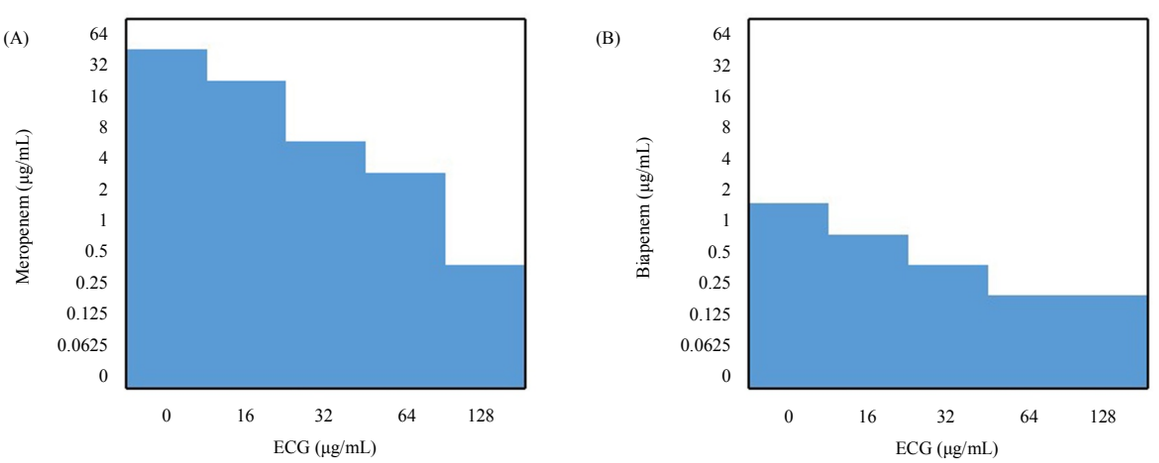

Figure 3. Microdilution checkerboard analysis of ECG and meropenem (A); and biapenem (B).

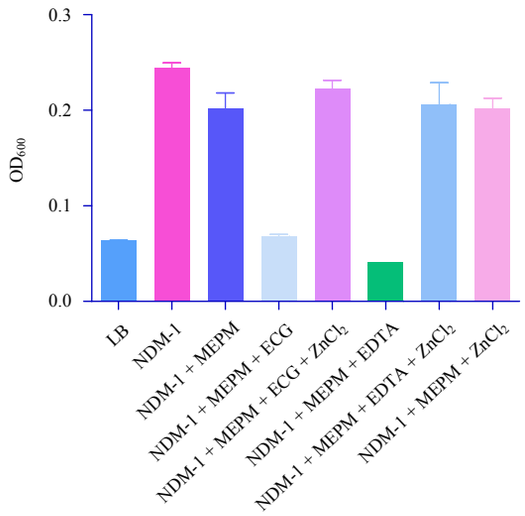

Figure 4. Supplement of exogenous zinc ion reverses the inhibitory effect of ECG on NDM-1.

the addition of ECG restored the activity of meropenem, resulting in growth suppression. However, the effect of ECG was reversed by supplement of exogenous zinc ion, suggesting that ECG inactivated NDM-1 probably *via* a zinc-related mechanism.

3.5. ECG shows relatively good safety both *in vitro* and *in vivo*

The potential toxicity of ECG was measured using Vero cell line and HepG2 human cell line. ECG showed low toxicity to both cell lines, with an IC_{50} value of 106.3 μM for Vero cells, and an IC_{50} value of 80.76 μM for HepG2 cells. Additionally, an acute toxicity study in mice treated with 500 mg/kg oral dose of ECG didn't show apparent abnormality. All these results indicated a relatively good safety of ECG.

4. Discussion

Since the NDM-1 was first described in New Delhi, *blaNDM-1* spread rapidly through plasmids and mobile genetic elements in various regions and countries of the world, including China[17]. According to statistics, the number of reported NDM-1 inhibitors is more than 500 up to now[8]. Based on mechanism, they can be roughly divided into three types: (i) interacting with zinc ion: these inhibitors usually produce an inhibitory effect *via* chelating or coordinating with zinc ion within the active pocket of NDM-1; (ii) interacting with critical amide acid residues: these inhibitors interact with critical amide acids, blocking the biological function of them or occupying active pocket in space; (iii) interacting with zinc ion and critical amide acids at the same time: seen as the most promising inhibitors, these inhibitors work on both zinc ion and amide acids, forming a stable effect[8].

We have previously constructed a high-throughput screening model for NDM-1. From 52 100 compounds, ECG is screened out as a potent inhibitor for NDM-1. The biological activity and action mode were partially elucidated in this article. As described above, ECG reversibly inactivated NDM-1, with an IC_{50} value of 4.48 μM. The interaction between ECG and NDM-1 was revealed by the fluorescence quenching experiment. The fluorescence of NDM-1 was mainly attributed to aromatic amide acids. ECG probably bound to the aromatic amide acids of NDM-1, changing the nonpolar environment towards the polar environment, subsequently quenching the fluorescence. Besides, the surface plasmon resonance experiment further confirmed this interaction.

Not only ECG exhibits an inhibitory effect at the enzyme level, but also it is able to restore the antibacterial efficacy of β-lactam antibiotics against NDM-1-producing bacteria. When co-treated with 32 μg/mL ECG, the MIC of piperacillin, cefoperazone, meropenem, and biapenem against engineered NDM-1 harboring strain was decreased to 2-fold, 4-fold, 4-fold, and 4-fold, respectively. Carbapenems have been

regarded as the most potent antibiotics against severe bacterial infection, including ESBL-produing infection[18,19]. Undoubtedly, carbapenem resistance poses threat to human health. In our present research, the synergism of ECG to meropenem and biapenem was noteworthy. When gradient ECG was added from 0 to 128 μg/mL, the MIC of meropenem ranged from 64 to 0.5 μg/mL, and the MIC of biapenem ranged from 2 to 0.25 μg/mL, correspondingly. This result suggested the possibility of ECG as an adjuvant of carbapenems.

Zinc ions on the active pocket are essential for the hydrolytic activity of NDM-1, while Zn1 coordinates with O atom of carbonyl, Zn2 coordinates N atom of lactam[20]. Among reported NDM-1 inhibitors, those which work by interacting zinc ions play a remarkable effect, such as Aspergillomarasmine A, a natural product originated from fungi, irreversibly inhibiting NDM-1 *via* removing the zinc ion[21]. However, zinc-dependent enzymes not only exist in bacteria, but also play a significant role in maintaining the normal physiological function of humans[22–24]. The selectivity and safety remain tough challenges to this zinc-targeted strategy. As shown above, the supplement of exogenous zinc ion almost entirely reversed the synergism of ECG on meropenem, suggesting that ECG probably worked on zinc ion facilitating NDM-1 inhibition. Notably, ECG exhibited low toxicity both *in vitro* and *in vivo*, and 500 mg/kg oral dose appeared to be non-lethal, which guaranteed a good safety profile of ECG.

Collectively, we preliminarily studied the activity and action mechanism of ECG as a novel NDM-1 inhibitor in the present study. According to our results, ECG reversibly and noncompetitively inactivated NDM-1 *via* a zinc-related mechanism, recovering several β-lactam antibiotics against resistant NDM-1-producing strain. The specific action sites and β-lactamase inhibition spectrum of it deserved further attention.

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References

[1] Breijyeh, Z.; Jubeh, B.; Karaman, R. Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. *Molecules*. **2020**, *25*, 1340.

[2] Fritzenwanker, M.; Imirzalioglu, C.; Herold, S.; Wagenlehner, F.M.; Zimmer, K.P.; Chakraborty, T. Treatment options for carbapenem-resistant gram-negative infections. *Dtsch. Arztebl. Int.* **2018**, *115*, 345–352.

[3] Nordmann, P.; Poirel, L. Epidemiology and diagnostics of carbapenem resistance in gram-negative bacteria. *Clin. Infect. Dis.* **2019**, *69*, S521–S528.

[4] Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. *World Health Organization*. http://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf.

[5] Bonomo, R.A.; Burd, E.M.; Conly, J.; Limbago, B.M.; Poirel, L.; Segre, J.A.; Westblade, L.F. Carbapenemase-producing organisms: a global scourge. *Clin. Infect. Dis.* **2018**, *66*, 1290–1297.

[6] Shi, C.; Chen, J.; Kang, X.; Shen, X.; Lao, X.; Zheng, H. Approaches for the discovery of metallo-β-lactamase inhibitors: A review. *ChemBiol. Drug Des*. **2019**, *94*, 1427–1440.

[7] Tooke, C.L.; Hinchliffe, P.; Bragginton, E.C.; Colenso, C.K.; Hirvonen, V.H.A.; Takebayashi, Y.; Spencer, J. B-lactamases and β-lactamase inhibitors in the 21st century. *J. Mol. Biol.* **2019**, *431*, 3472–3500.

[8] Linciano, P.; Cendron, L.; Gianquinto, E.; Spyrakis, F.; Tondi, D. Ten years with new Delhi metallo-β-lactamase-1 (NDM-1): from structural insights to inhibitor design. *ACS Infect. Dis.* **2019**, *5*, 9–34.

[9] Liu, B.; Trout, R.E.L.; Chu, G.H.; McGarry, D.; Jackson, R.W.; Hamrick, J.C.; Daigle, D.M.; Cusick, S.M.; Pozzi, C.; De Luca, F.; Benvenuti, M.; Mangani, S.; Docquier, J.D.; Weiss, W.J.; Pevear, D.C.; Xerri, L.; Burns, C.J. Discovery of taniborbactam (VNRX-5133): a broad-spectrum serine- and metallo-β-lactamase inhibitor for carbapenem-resistant bacterial infections. *J. Med. Chem.* **2020**, *63*, 2789–2801.

[10] Fu, B.; Zeng, Q.H.; Zhang, Z.T.; Qian, M.Y.; Chen, J.C.; Dong, W.L.; Li, M. Epicatechin gallate protects HBMVECs from ischemia/reperfusion injury through ameliorating apoptosis and autophagy and promoting neovascularization. *Oxidative Med. Cell. Longev.* **2019**, *2019*, 7824684.

[11] Esmaeelpanah, E.; Razavi, B.M.; Vahdati Hasani, F.; Hosseinzadeh, H. Evaluation of epigallocatechin gallate and epicatechin gallate effects on acrylamide-induced neurotoxicity in rats and cytotoxicity in PC 12 cells. *Drug Chem. Toxicol.* **2018**, *41*, 441–448.

[12] Sánchez-Tena, S.; Alcarraz-Vizán, G.; Marín, S.; Torres, J.L.; Cascante, M. Epicatechin gallate impairs colon cancer cell metabolic productivity. *J. Agric. Food Chem.* **2013**, *61*, 4310–4317.

[13] Satsu, H.; Awara, S.; Unno, T.; Shimizu, M. Suppressive effect of nobiletin and epicatechin gallate on fructose uptake in human intestinal epithelial Caco-2 cells. *Biosci. Biotechnol. Biochem.* **2018**, *82*, 636–646.

[14] Stevens, C.S.; Rosado, H.; Harvey, R.J.; Taylor, P.W. Epicatechin gallate, a naturally occurring polyphenol, alters the course of infection with β-lactam-resistant Staphylococcus aureus in the zebrafish embryo. *Front Microbiol.* **2015**, *6*, 1043.

[15] Huang, C.C.; Wu, W.B.; Fang, J.Y.; Chiang, H.S.; Chen, S.K.; Chen, B.H.; Chen, Y.T.; Hung, C.F. (–)-Epicatechin-3-gallate, a Green Tea Polyphenol Is a Potent Agent Against UVB-induced Damage in HaCaT Keratinocytes. *Molecules*. **2007**, *12*, 1845–1858.

[16] Han, J.X.; Xiao, C.L.; Gan, M.L.; Li, X.H.; Wang, Y.; Zheng, J.Y.; Li, D.S.; Liu, C.C.; Guan, Y.; Meng, J.Z.; Huang, S.C.; Liu, Y.S. IMB-XH1 identified as a novel inhibitor of New Delhi metallo-β-lactamase-1. *J. Chin. Pharm. Sci*. **2019**, *28*, 238–246.

[17] Farhat, N.; Khan, A.U. Evolving trends of New Delhi Metallo-betalactamse (NDM) variants: a threat to antimicrobial resistance. *Infect. Genet. Evol.* **2020**, *86*, 104588.

[18] Iovleva, A.; Doi, Y. Carbapenem-Resistant Entero-bacteriaceae. *Clin. Lab. Med*. **2017**, *37*, 303–315.

[19] Tamma, P.D.; Rodriguez-Bano, J. The Use of Noncarbapenem β-Lactams for the Treatment of Extended-Spectrum β-Lactamase Infections. *Clin Infect Dis*. **2017**, *64*, 972–980.

[20] Groundwater, P.W.; Xu, S.; Lai, F.; Váradi, L.; Tan, J.; Perry, J.D.; Hibbs, D.E. New Delhi metallo-β-lactamase-1: structure, inhibitors and detection of producers. *Future Med. Chem*. **2016**, *8*, 993–1012.

[21] King, A.M.; Reid-Yu, S.A.; Wang, W.; King, D.T.; De Pascale, G.; Strynadka, N.C.; Walsh, T.R.; Coombes, B.K.; Wright, G.D. AMA overcomes antibiotic resistance by NDM and VIM metallo-β-lactamases. *Nature*. **2014**, *510*, 503–506.

[22] Porter, N.J.; Christianson, D.W. Structure, mechanism, and inhibition of the zinc-dependent histone deacetylases. *Curr. Opin. Struct. Biol.* **2019**, *59*, 9–18.

[23] Bernstein, K.E.; Khan, Z.; Giani, J.F.; Cao, D.Y.; Bernstein, E.A.; Shen, X.Z. Angiotensin-converting enzyme in innate and adaptive immunity. *Nat. Rev. Nephrol.* **2018**, *14*, 325–336.

[24] Wang, X.; Khalil, R.A. Matrix metalloproteinases, vascular remodeling, and vascular disease. *Adv. Pharmacol.* **2018**, *81*, 241–330.

一种新型NDM-1抑制剂: (–)-表儿茶素没食子酸酯

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摘要: NDM-1导致的β-内酰胺类抗生素耐药问题已成为威胁全球公共卫生的重大危机。我们前期经筛选发现(–)-表儿茶素没食子酸酯是一种有效的NDM-1抑制剂, 本文进一步探讨了其抑制活性和作用方式。结果显示, (–)-表儿茶素没食子酸酯以一种可逆的、非竞争性的模式抑制NDM-1的活性, IC_{50}值为4.48 μM。(–)-表儿茶素没食子酸酯能有效恢复多种β-内酰胺类抗生素对NDM-1菌的抑菌活性, 特别是对于碳青霉烯类抗生素有较显著的作用。锌离子回补实验结果提示, (–)-表儿茶素没食子酸酯发挥作用可能与对锌离子的影响有关, 然而不同于传统螯合剂, 它在体内外均展现出较低的毒性。综上所述, 我们的结果显示(–)-表儿茶素没食子酸酯能够协助碳青霉烯类抗生素抑制NDM-1产生菌, 是一种具有潜力的NDM-1抑制剂。

关键词: NDM-1; (–)-表儿茶素没食子酸酯; 抑制剂; 抗生素耐药