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Drug Repurposing: Research Progress of Niclosamide and Its Derivatives on Antibacterial Activity

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Abstract: The development of antibiotic resistance complicates the treatment of infectious diseases and is a global public health threat. However, drug repurposing can address this resistance issue and reduce research and development costs. Niclosamide is a salicylanilide compound approved by the Food and Drug Administration (FDA), and it has been used clinically for treating parasitic infections for many years. Recent studies have shown that niclosamide can inhibit bacterial and fungus activity by affecting the quorum sensing system, biofilm formation, cell membrane potential, and other mechanisms. Here, we discuss recent advances in the antimicrobial applications of niclosamide and its derivatives to provide new perspectives in treating infectious diseases.

Keywords: antimicrobial resistance, multi-drug resistance, niclosamide, a derivative, drug repurposing

Introduction

Antibiotics have been used to treat bacterial infections for over 70 years and have been the most important class of drugs since the twentieth century.[1] However, excessive or inappropriate use of antibiotics has led to the emergence of antimicrobial resistance (AMR) and the production of multidrug-resistant (MDR) bacteria in the clinic, in animals, and even in the environment.[2] Experts estimate that AMR could force 24 million people into extreme poverty by 2030 and that MDR bacterial infections could cause 10 million deaths per year worldwide by 2050.[3] The search for new antibiotics for clinical use is becoming increasingly challenging due to the significant costs associated with drug development and the rapid emergence of drug resistance.[4] Drug repurposing is a creative strategy to address this problem. This strategy has been successfully applied to the discovery of potential treatments for a wide range of diseases, including infectious diseases. In recent years, it has been shown that, among non-antimicrobial drugs, auranofin[5] and ebselen[6] are active against many kinds of bacteria by inhibiting thioredoxin reductase (TrxR), while NIC kills microorganisms by inhibiting mechanisms such as quorum sensing systems and biofilm formation. It has attracted wide attention due to NIC's strong antibacterial activity, inhibit many kinds of bacteria, and the fact that it is not easy to develop drug resistance.

NIC, a clinical antiparasitic drug approved by the FDA in 1982, works as a hydrogen ionophore that plays a role. NIC inhibits adenosine triphosphate (ATP) production through oxidative phosphorylation and uncoupling of electron transfer, leading to parasite death.[7] Over the past decades, evidence has shown that NIC can also act on other targets, such as the Wnt/β-catenin, mTOR, and JAK/STAT3 signaling pathways.[8] These pathways play an essential role in developing and progressing many diseases. The versatility of NIC has been increasingly demonstrated, while its potential in treating infectious diseases has been progressively discovered. In this paper, by searching for relevant literature in globally

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recognized databases such as PubMed, Web of Science, and Google Scholar. For the first time, the antibacterial and antifungal activities and mechanisms of action of NIC and its derivatives are reviewed. In addition, a brief summary of the application of NIC in other medical fields was provided. Finally, the shortcomings and perspectives of NIC and its derivatives are discussed. New ideas for the pharmaceutical reuse of NIC are provided.

History, Structure, Metabolism, and Applications of NIC

NIC, also known as Bayluscide, is an odorless, water-insoluble, yellowish-white crystalline powder. In the late 1950s, Bayer first discovered through screening that NIC could control Schistosoma mansoni transmission and developed and marketed it as a molluscicide.[9] In 1960, Bayer scientists discovered that it also played an essential role in controlling parasitic infections in humans. In 1962, it was marketed for human use under the trade name Yomesan.[10] The FDA added NIC to the World Health Organization (WHO) list of essential drugs in 1982.[11]

Structurally, NIC belongs to a large group of lipophilic, weakly acidic molecules called salicylanilides (Figure 1a).[12] NIC is a product formed through the condensation of the carboxy group of 5-chlorosalicylic acid with the amino group of 2-chloro-4-nitroaniline. The molecular formulation of NIC is C13H8Cl2N2O4 with a molecular weight of 327.12 Dalton (Da)(Figure 1b).[13]

A clinical trial showed that a single dose of 2000 mg NIC daily for 5–7 days in patients weighing more than 56 kg effectively treated infections caused by *H. nana, Taenia saginata*, or *Diphyllobothrium latimi*.[14] In addition, NIC has even been used in pediatric patients at a dose of 80 mg/kg, and no adverse toxicity has been reported.[15] NIC is partially absorbed in the intestinal tract of humans and animals and then rapidly excreted through the kidneys, making it highly safe and tolerated in controlling parasitic infections.[12]

In recent years, high-throughput drug screening studies have identified NIC also as a potential drug with antitumor[16] and antiviral[17] effects et al (Table 1). Over the past fifteen years, research on NIC has gradually become a hot topic of great interest in the field of medicine, as reflected in the significant increase in the number of publications on the drug (Figure 2).

Antimicrobial Activity

Gram-Positive Bacteria

Infections caused by Gram-positive bacteria in healthcare and community settings are a severe problem, especially with the prevalence of MDR bacteria. The WHO updated the list of bacteria in urgent need of antibiotic treatment in May 2024, and *methicillin-resistant Staphylococcus aureus (MRSA)* and *vancomycin-resistant Enterococcus faecalis (VRE)* remain in the high-priority category.[47]

Staphylococcus Aureus

Staphylococcus aureus (S. aureus) is one of the major pathogens associated with drug resistance and commonly colonizes

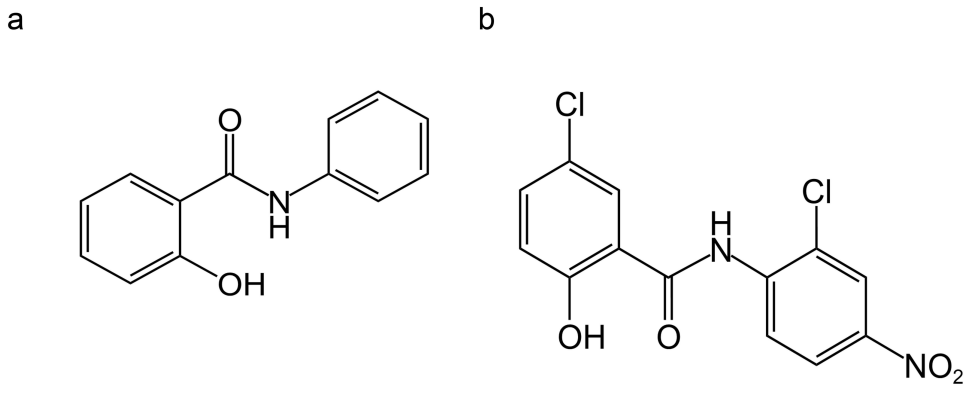

Figure 1 (**a**)The structure of salicylamide; (**b**)The structure of niclosamide.

Table 1 Other Applications and Mechanisms of NIC

Apply	Diseases	Mechanism	Refs
Antitumor	Leukemia	Inhibits cell growth and induces apoptosis by increasing ROS levels and activating cleaved cysteine-3, LC3B, and TP53	[18,19]
	Triple-negative breast cancer	Inhibition of the amino acid transport proteins SLC38A5 and SLC7A11 expression, decreased glutathione levels, increased lipid peroxidation, and induced TNBC cell iron death	[20]
	Cholangiocarcinoma	Reduced the mitochondrial membrane potential of two CCA cell lines, KKU213A and KKU-100, leading to ATP depletion	[21]
	Pancreatic cancer	Induced ubiquitylation β-catenin of degradation synergistically enhances the antitumor effects of gemcitabine	[22]
	Ovarian cancer	Inhibits high expression of FXR1 or IGF2BP2 in ovarian cancer cells and reduces cell viability, adhesion, and migration	[23]
	Malignant melanoma	Inhibition of TGF-β-stimulated EMT signaling and metastasis	[24]
	Endometrial cancer	Inhibits the AKT/mTOR signaling pathway and induces apoptosis and autophagy in endometrial cancer cells	[25]
	Osteosarcoma	Inhibition of β-catenin attenuates osteosarcoma resistance to chemotherapeutic agents and inhibits osteosarcoma cell proliferation	[26]
	Colorectal cancer	Reduces PXR expression and CSC numbers in colorectal cancer cell lines and acts synergistically with chemotherapeutic agents to prevent in vivo CSC chemoresistance and tumor recurrence	[27]
	Non-small cell lung cancer	Induction of NSCLC cell death and autophagy through the AMPK/AKT/mTOR pathway	[28]
Antiviral	SARS-CoV-2	Reduced cytoplasmic translocation of HuR and reduced *CD147* glycosylation	[29,30]
	Zika virus	Inhibits ZIKV replication by eliminating fragmented mitochondria	[31]
	Hepatitis E virus	Inhibition of NFκB signaling	[32]
	Respiratory syncytial virus	Decreased AKT pro-survival protein, activated lysed caspase-3 and poly ADP-ribose polymerase	[33]
	HIV-1	Affects the AMPK-mTORC1 pathway	[34]
Antifibrosis	Fibroblast fibrosis	Blocks the MAPK-ERK1/2 signaling pathway, thereby inhibiting TGF-β1-induced fibrosis in human Tenon's fibroblasts	[35]
	Hyperplasia of prostate	Inhibits fibrosis through Wnt/β-catenin signaling	[36]
	Renal fibrosis	Inhibition TGF-β/Smad3 signaling	[37]
	Hepatic fibrosis	Affects NOTCH paths, Wnt paths	[38]
Cardiovascular disease	Aortic valve calcification	Inhibition of AKT and ERK activation to target the oxidative stress-mediated GSK-3β/β-catenin signaling pathway	[39]
	Vascular calcification	Inhibits the Wnt signaling pathway and reduces Runx2 expression	[40]
	Heart failure	Inhibition of collagen secretion by cardiac fibroblasts and reduction of the serum inflammatory mediator IL-6	[41]
	Atherosclerosis	Downregulation of somatic LOX-1 expression and modification of carotid plaque composition in mice	[42]

(*Continued*)

Table 1 (Continued).

Apply	Diseases	Mechanism	Refs
Other	anti-inflammatory	Inhibition of ANO1 and ANO6 and reduction of intracellular Ca^{2+} levels	[43]
	Diabetic nephropathy	Inhibition of Wnt/β-catenin pathway, RAAS expression of multiple genes	[44]
	Acute liver failure	Regulation of the DMT1/FPN1 iron transport protein axis inhibits iron loading	[45]
	Amyotrophic lateral sclerosis	Inhibit ALS's dysregulated various molecular pathways, such as STAT3 and mTOR	[46]

the human skin and respiratory tract.[48] When the host's defense mechanisms are compromised, it can lead to various types of infections, including skin infections, pneumonia, osteomyelitis, and endocarditis.[48] It has been reported that *S. aureus* has developed varying degrees of resistance to various antibiotics such as β-lactams, aminoglycosides, and tetracyclines.[49] In addition, *S. aureus* can form biofilms that slow down the metabolism of the bacteria, resulting in more excellent resistance of the bacteria within the membrane to antibiotics.[50]

Rajamuthiah et al[51] determined the in vitro antimicrobial activity of NIC against *S. aureus* strains (*MRSA* MW2, *Newman*, RN4220, RN6390, USA100, USA300, USA400) using agar disk diffusion and broth microdilution methods. Among them, the MIC of *MRSA* MW2 was 0.125μg/mL, and the MIC of the remaining strains was less than 0.0625μg/mL. Sheep erythrocytes were treated with serially diluted NIC for one hour, and the measurement of absorbance at 540 nm indicated that NIC did not cause erythrocyte hemolysis, even at the maximum concentration tested. Subsequently, the researchers established a Caenorhabditis elegans infection model. They found that NIC was as effective as vancomycin in prolonging the survival time of infected worms when the concentration of NIC reached 0.78 μg/mL.

Torres et al[52] screened 1280 compounds from the Prestwick chemical library at a fixed concentration of 3.27μg/mL. One hundred and four drugs were found to be effective in removing more than 55% of airborne microbes from *MRAS* TCH1516. Subsequent biofilm testing showed that NIC had antibacterial activity against pre-formed biofilms, with a 1 log unit reduction in the number of viable bacteria in the biofilm after only two drug treatments.

Gwisai et al[53] developed NIC coatings on various surfaces, including medically relevant aluminum, stainless steel, and titanium, using solvent casting. The activity of NIC coatings on *S. aureus* 25923, *MRSA* MW2, and *S. epidermidis* 9142 was evaluated. When the concentration on the coated surface is as low as 1.6×10^{-2}μg/mm^2, the adhesion of these bacteria is effectively prevented. In addition, NIC inhibited the growth of *S. aureus, MRSA* (MIC 0.156–0.313μg/mL), and *S. epidermidis* (MIC 0.063–0.125μg/mL) at MIC concentrations lower than vancomycin. At the same time, it prevents the formation of *staphylococcal* biofilms and reduces their number at the corresponding MIC concentration. This study demonstrates that NIC has the potential for antimicrobial device coatings.

Zhang et al[54] determined the activity of NIC against *S. aureus* ATCC 29213, *MRSA*, and clinical isolates *MRSA*-1 (DL-5F2) and *MRSA*-2 (DN-65) with MIC of 0.06–0.125μg/mL. After 15 days of continuous passaging against *S. aureus* at sub-MIC of NIC, no resistant mutants of the strain were detected, compared to the control group, the MIC of

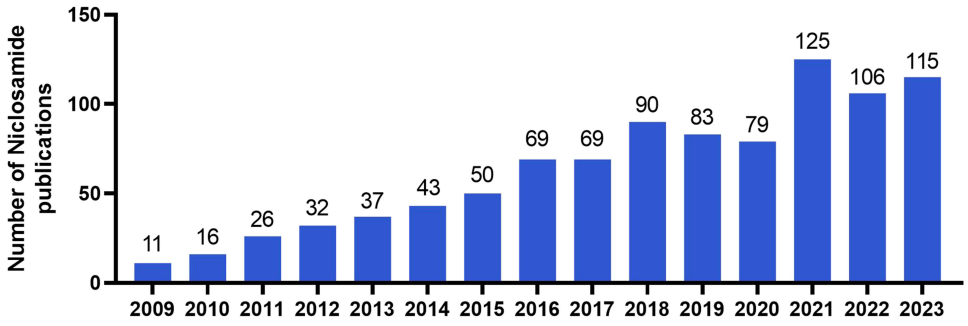

Figure 2 The number of NIC papers published since 2009. The data were obtained from PubMed using the search term "NIC"and fixing the dates from January 1st to December 31st of each indicated year.

ciprofloxacin and oxacillin sodium increased about 14-fold against *S. aureus*, suggesting the superiority of NIC in combating multi-drug resistance. After mice were infected with *S. aureus*, the untreated group of mice showed significant abscesses and redness at the site of infection. In contrast, epidermal cysts were significantly reduced in groups of mice treated with different doses of NIC and ciprofloxacin. In particular, no significant abscesses were observed at the site of infection in mice treated with 10 mg/kg bw and 20 mg/kg bw NIC or ciprofloxacin. Pathological section analysis revealed a significant reduction in the number of pyogenic and inflammatory cells at the site of infection in mice in the treatment group.

Vazquez et al[55] investigated the activity of NIC against *MSSA* ATCC 25923, *MRSA* ATCC 33591, *MSSE* O47, and *MRSE* ATCC 35984 by disk diffusion assay, quantification of bacteria within the biofilm on the catheter surface, and observation of bacterial growth in airborne microbes in liquid media. The results showed that the MIC of the NIC-loaded catheter segments was in the range of 0.0625 to 0.5 μg/mL for the strains tested. The in vivo efficacy of catheters loaded with 2% and 5% NIC was evaluated against *MSSA* and *MRSA* strains by constructing a mouse BAI model. The results showed that both NIC-containing catheters exhibited the best results on day 14. Catheter segments loaded with 2% and 5% NIC had significantly lower bacterial loads of 4.29 and 3.40 LogCFU/catheter, respectively ($p < 0.001$ and $p < 0.0001$), compared to catheter segments not loaded with NIC, which had a bacterial load of 6.06 LogCFU/catheter ($p < 0.0001$). In addition, the drug concentrations released are not cytotoxic at the plasma level. This research is expected to lead to the future development of novel functionalized medical devices (such as heart valves, urinary catheters, endotracheal tubes, prosthetic implants, etc.) to prevent or treat *staphylococcal* infections.

Enterococcus

Enterococci usually colonize patients' skin, intestines, and urinary systems and can survive for long periods in the hospital environment.[56] *VRE* is a subtype of enterococci resistant to several antibiotics.[57] It is more challenging to treat than infections caused by strains sensitive to vancomycin, with a mortality rate as high as 64%.[58] Therefore, reducing *VRE* colonization in patients can reduce the incidence of infection, help control treatment costs, and improve cure rates.

Mohammad et al[59] tested the MIC of NIC against 15 strains of *E. faecium* (including *VRE*) in clinical isolates with MIC of 1–8μg/mL and antimicrobial activity similar to linazolamide and ramoplanin using the broth microdilution method. *E. faecium* did not develop resistance to NIC after several consecutive passages, and the MIC only doubled. In the mouse infection model, NIC reduced *VRE* load in the cecum by 2.4-$\log_{10}$ and in the ileum by 1.8-$\log_{10}$ after only eight days of treatment at the safe dose range, which was significantly effective in reducing bacterial load in the mouse gut and superior to linazolamide. Upon further evaluation, NIC is expected to serve as a novel decolonizing agent to inhibit *VRE* intestinal infections.

Corynebacterium Striatum

Corynebacterium striatum (C. striatum) is an emerging MDR pathogen associated with hospital-acquired infections. It is commonly isolated from various bioregions, such as wounds, bone biopsies, and bloodstreams.[60] Between 1976 and 2020, several countries reported outbreaks of MDR *C. striatum*.[61] In recent years, *C. striatum* has shown resistance to antibiotics such as β-lactams, macrolides, and aminoglycosides, posing a challenge to clinical treatment.[62]

Folliero et al[63] investigated the antimicrobial activity of NIC against 20 clinical isolates of *C. striatum* with an MIC_{90} of 0.39μg/mL by Kirby–Bauer test, broth microdilution method, and biofilm degradation test. Meanwhile, crystal violet and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assays showed that NIC had vigorous degradation activity on the bacterial biofilm matrix. Subsequently, the toxic effect of NIC on human immortalized keratinocytes (HaCaT) was evaluated by MTT, and NIC did not cause substantial changes in cell viability with a percentage of living cells of 80% in the concentration range of 0.02–3.12μg/mL.

Clostridium Difficile

Clostridium difficile (C. difficile) is an obligate anaerobic, spore-bearing, toxin-producing Gram-positive bacterium found primarily in soil and the intestinal tract.[64] Toxins released during infection cause inflammation and lesions in the intestinal tract, resulting in diarrhea and abdominal pain.[65] Following broad-spectrum antibiotics, the balance of normal

gut microbiota is disrupted, and the diversity of gut microorganisms is reduced, leading to the development of *C. difficile*-associated disease (CDAD).[66]

Gooyit et al[67] evaluated the in vitro antimicrobial activity of NIC against the strongly virulent, multidrug-resistant *C. difficile* strain 630 and the BI/NAP1/027 highly virulent *C. difficile* strain 4118 by broth microdilution method, and the results showed that its MIC was similar to that of metronidazole (0.13–0.25μg/mL) and vancomycin (0.5–2μg/mL). Subsequently, the MIC_{90} of NIC against 16 clinical isolates of *C. difficile* was detected to be 2μg/mL. In addition, NIC does not have a destructive effect on intestinal commensal bacteria (including Bacteroidaceae, Bifidobacterium, and Lactobacillus spp.) and maintains the balance of intestinal tract normal flora, which helps to inhibit *C. difficile toxin*-induced disease.

Gram-Negative Bacteria

Gram-negative bacteria are one of the essential pathogens in hospital-acquired infections.[68] They cause about 45–70% of cases of ventilator-associated pneumonia (VAP) and about 20–30% of cases of catheter-associated bloodstream infections. In addition, other infections are also common in the ICU setting, such as surgical site infections or urinary tract infections.[69] Hospital-acquired gram-negative pathogens gradually show high antibiotic resistance levels.[70]

Pseudomonas Aeruginosa

Pseudomonas aeruginosa (P. aeruginosa) is a common opportunistic human pathogen that can cause high morbidity and mortality in humans with weakened immune systems or hospitalized patients.[71] The production of *P. aeruginosa* biofilm and virulence factors is responsible for the prolonged difficulty in curing the infection.[72] In addition, *P. aeruginosa* can acquire new resistance genes through horizontal gene transfer, making it more resistant.[73] Therefore, studying drugs targeting biofilms and significant virulence factors is essential for effectively treating *P. aeruginosa* infections.

Imperi et al[74,75] investigated the effect of NIC on *P. aeruginosa* in vitro and in vivo. The results showed that although NIC reduced the in vitro activity of *P. aeruginosa* and the pathogenicity of *P. aeruginosa* against the large wax borer, the inhibition of bacterial growth was not significant.

Costabile et al[76] prepared an inhalable nanosuspension (T80_10) of NIC that inhibited *P. aeruginosa* activity and reduced biofilm production at concentrations ranging from 0.82 to 3.27μg/mL. The suspension was not found to significantly reduce bronchial epithelial cell viability in vitro. To investigate the acute toxic response of NIC (T80_10) suspension on rats and their lung injury status after 24 hours, the researchers evaluated the expression levels of inflammatory markers iNOS and COX-2 enzymes in lung homogenates of rats in each group within 24 hours by photodensitometry. The study results showed that no significant differences in iNOS expression and COX-2 enzyme expression were detected in lung homogenate samples from all experimental groups of rats, indicating no acute toxic effects of NIC. When NIC is administered as an inhalable nanosuspension, it will potentially treat pulmonary infections with *P. aeruginosa*.

Helicobacter Pylori

Helicobacter pylori (H. pylori) is a Gram-negative helical pathogen associated with the human gastric mucosa and is one of the major causes of gastric ulcers and gastritis.[77] It is estimated that more than 50% of the world's population is infected with *H. pylori*, and the prevalence of this infection is exceptionally high in some resource-poor developing countries.[78] Prolonged and unregulated antibiotic use results in single-base changes in the *H. pylori* genome, which makes the strain more resistant to antibiotics.[79]

Tharmalingam et al[80] demonstrated the inhibitory effect of NIC on *H. pylori* ATCC 49503, with an MIC of 0.25μg/mL and an MBC of 0.5μg/mL. As determined by the checkerboard assay, NIC is partially synergistic with metronidazole and proton-pump inhibitor against *H. pylori* and has no antagonistic effect. Studies have shown that NIC is stable at an acidic pH and does not develop drug resistance to *H. pylori*. In the large wax borer infection model, Larvae were tested for survival every 24 hours and were judged dead if they did not respond to environmental stimuli. Larvae survived up to 70% after NIC treatment compared to the untreated group ($p < 0.0001$).

Fungus

The prevalence of fungus infections has progressively increased over the past three decades, resulting in more than one million deaths yearly.[81] In the United States alone, diseases caused by fungus infections cost more than $7.2 billion annually.[82] In addition, current antifungal drug options remain limited and are threatened by the continued emergence of drug-resistant fungus strains.[83]

Candida Albicans

Candida albicans (C. albicans) are part of the normal microbiota and infect target organs by overgrowth or hemorrhagic spread from colonization sites when the body's defenses are compromised.[84] The *C. albicans* biofilm is a complex, multilayered community of cells, and this structure makes it resistant to almost all classes of antifungal drugs. It adheres to the surface of medical devices and host tissues and can protect the strain from the environment, innate immune cells, and antifungal drugs, making treatment more complex.[85]

Garcia et al[86] screened drugs against *C. albicans* from chemical libraries and found that NIC inhibited *C. albicans* growth at 0.16–1.64μg/mL concentrations. Anti-biofilm activity was observed at a concentration of 0.33μg/mL, and more than 15% of biofilms were inhibited at a concentration of 1.64μg/mL. Meanwhile, the data suggest that NIC attenuates the destruction of human colonic epithelial cells by *C. albicans*.

Sutar et al[87] based on nanotechnology[88,89] developed highly NIC-loaded nanoparticles (NIC-EPO-NP) that effectively inhibited the growth of *C. albicans* SC5314 at concentrations as low as 0.5μg/mL. It has been shown to penetrate mature biofilms completely and at lower concentrations (approximately 0.5–2μg/mL) to inhibit fluconazole-resistant *C. albicans* biofilms (MIC $_{FCZ}$ >128μg/mL) by more than 50%. To evaluate the effect of NCL-EPO-NP on *C. albicans* in vivo, the researchers mixed NCL-EPO-NP into an in situ gel formulation consisting of 20% w/v P407 and 1% w/v poloxamer 188. The excellent biocompatibility of the gel formulation facilitates[90] its direct entry into the mucosal organs of the mice. Subsequently, an oropharyngeal candidiasis (OPC) mouse model was established, and treatment with NIC-EPO-NP gel resulted in an almost 1 log reduction in fungal load compared to untreated controls. NCL-EPO-NP gel reduced the fungal load in the oral mucosa by 10-fold during treatment of a fluconazole-resistant *C. albicans* mouse infection model. In addition, the researchers found that 20 μg/mL of NCL-EPO-NP gel almost eliminated vaginal infectionIn conclusion, the NCL-EPO-NP gel for treating candidiasis has also shown significant results in vivo.s in fluconazole-resistant mice in an independent mouse model of vulvovaginal candidiasis.

Madurella Mycetomatis

Madurella mycetomatis (M. mycetomatis) is commonly associated with the fungus mycetomatosis, which can lead to chronic infections of the skin and deep tissues and may eventually affect muscles, tendons, and bones.[91] *M. mycetomatis* commonly infects the foot and is known as mycetoma, and ketoconazole and itraconazole have been approved for treating this disease.[92] Due to the low cure rate of clinical antifungal medications, amputation may be the only current treatment for this disease.[93]

Mahmoud et al[94] tested a panel of nitro-aromatics by in vitro assay and found that NIC exhibited in vitro activity against *M. mycetomatis* SO1 and *M. mycetomatis* CBS131320 with a MIC of 0.78 and 1.6μg/mL, respectively. NIC is expected to be a candidate for repurposing in mycetoma.

Sporothrix Genus

The *Sporothrix genus* is divided into three main taxa: *S. brasiliensis, S. globosa*, and *S. schenckii*,[81] which have had a wide global distribution in the last two decades.[82] The choice of therapeutic agents following *Sporothrix genus* infection is limited, especially for *S. brasiliensis*, which may be ineffective for conventional treatment.[83] In addition, the preferred drug, itraconazole, is not recommended for use in patients with liver disease, children, or pregnant women.[95]

Ramos et al[96] evaluated the activity of NIC against 18 clinical isolates of *Sporothrix* using the broth microdilution method. The results showed that NIC was effective against all *S. brasiliensis* strains, with a mean MIC of 0.4μg/mL, but it was not effective against *S. schenckii* and *S. globosa* strains (MIC > 6.54μg/mL). Although NIC was low cytotoxic to

human keratinocytes at a concentration of 2.56 μg/mL, 93% of *S. brasiliensis* strains were inhibited at concentrations well below the toxic concentrations.

In conclusion, NIC has good inhibitory activity against Gram-positive bacteria and fungi and poor activity against Gram-negative bacteria (Table 2).

Table 2 Antimicrobial Activity of NIC

Species	Strain	Method	Effect(μg/mL)	Mechanism	Refs
Gram-positive bacteria	*S. aureus* ATCC 29213	The agar dilution method	MIC=0.06	Inhibit the secretion of α-HL, Inhibit biofilm formation, Destruction of the cell wall	[54]
	MRSA MW2	Broth microdilution method	MIC=0.125	Inhibit the secretion of α-HL, Inhibit biofilm formation, Destruction of the cell wall	[51]
	MRSA ATCC 43300	The agar dilution method	MIC=0.06	Inhibit the secretion of α-HL, Inhibit biofilm formation, Destruction of the cell wall	[54]
	MRSA DL-5F2	The agar dilution method	MIC=0.125	Inhibit the secretion of α-HL, Inhibit biofilm formation, Destruction of the cell wall	[54]
	MRSA DN-65	The agar dilution method	MIC=0.125	Inhibit the secretion of α-HL, Inhibit biofilm formation, Destruction of the cell wall	[54]
	S. epidermidis 9142	Broth microdilution method	MIC=0.063–0.125	Not reported	[53]
	E. faecalis	Broth microdilution method	MIC=1-8	Not reported	[59]
	C. striatum	Broth microdilution method	MIC_{90}=0.39	Inhibit biofilm formation, Destruction of the cell wall	[63]
	C. difficile	Broth microdilution method	MIC_{50}=1; MIC_{90}=2	Affects bacterial cell membrane potential	[67]
Gram-negative bacteria	*P. aeruginosa* 14	Broth microdilution method	MIC>64	Suppress quorum sensing systems	[51]
	A. baumannii ATCC 17978	Broth microdilution method	MIC>64	Not reported	[51]
	K. pneumoniae ATCC 77326	Broth microdilution method	MIC>64	Not reported	[51]
	H. pylori ATCC 49503	Broth microdilution method	MIC=0.25; MBC=0.5	Affects bacterial cell membrane potential	[80]
Fungus	*C. albicans*	Broth microdilution method	MIC=0.16–1.64	Inhibit biofilm formation	[86]
	M.mycetomatis SO1	Broth microdilution method	MIC=0.78	Not reported	[94]
	M.mycetomatis CBS131320	Broth microdilution method	MIC=1.6	Not reported	[94]
	S. brasiliensis	Broth microdilution method	MIC=0.2–3.27	Not reported	[96]

Synergistic Effects of NIC with Antibiotics

Combination therapy is an effective treatment strategy for MDR bacteria.[97] In conventional treatment, antibiotics and antibiotic combinations are often used clinically to treat MDR bacterial infections. In recent years, there has been evidence that combining adjuvants and antibiotics may be another viable approach to treating MDR bacteria.[98] Adjuvants are compounds without bacteriostatic activity.[99] When used in combination with antibiotics to enhance the bactericidal effect of antibiotics through a variety of mechanisms, including interference with antibiotic-inactivating enzymes, membrane stability, or efflux pumps, and represent a complex but promising therapeutic strategy. In this strategy, the adjuvant and the antibiotic are serially diluted in multiplicity and mixed in different concentration combinations. After incubation with the bacterial solution for 18–24h, the MIC of the single drug and the combination of the two drugs are measured (Figure 3), and the fraction of inhibitory concentration index (FICI) is calculated using the formula. The formula for calculating FICI is as follows:

FICI = MIC at the combination of Drug A/MIC at Drug A alone + MIC at the combination of Drug B/MIC at Drug B alone (FICI ≤ 0.5, synergistic; FICI > 0.5–4.0, additive; FICI > 4.0, ntagonistic).

Domalaon et al[100] screened 31 kinds of antibiotics for clinical use and determined their in vitro activity against *P. aeruginosa* PAO1 jointly with NIC. When NIC (1μg/mL) was combined with colistin (0.125μg/mL), there was better synergistic activity with a fraction of FICI of 0.127. In contrast, NIC alone showed no significant activity against the *P. aeruginosa* PAO1 strain, with a MIC of up to 512μg/mL. In a rat model of renal ischemia/reperfusion injury, NIC significantly improved renal function, suggesting that it may also attenuate the nephrotoxicity of colistin.

Ayerbe et al[101] screened NIC in combination with colistin can enhance the inhibitory activity of colistin against Col-S *A. baumannii* ATCC 17978, 13 strains of clinical Col-R *A. baumannii*, Col-S *K. pneumoniae* CECT 997, one Col-S and two Col-R clinical *K. pneumoniae*. When colistin was used alone, the MIC of resistant strains ranged from 32 to >256 μg/mL, while the MIC of the sensitive strain was 0.5μg/mL. When NIC was used alone, the MIC ranged from 2 to 131 μg/mL for *A. baumannii* and 131 to >262 μg/mL for *K. pneumoniae*. The growth curve results showed that 0.65μg/

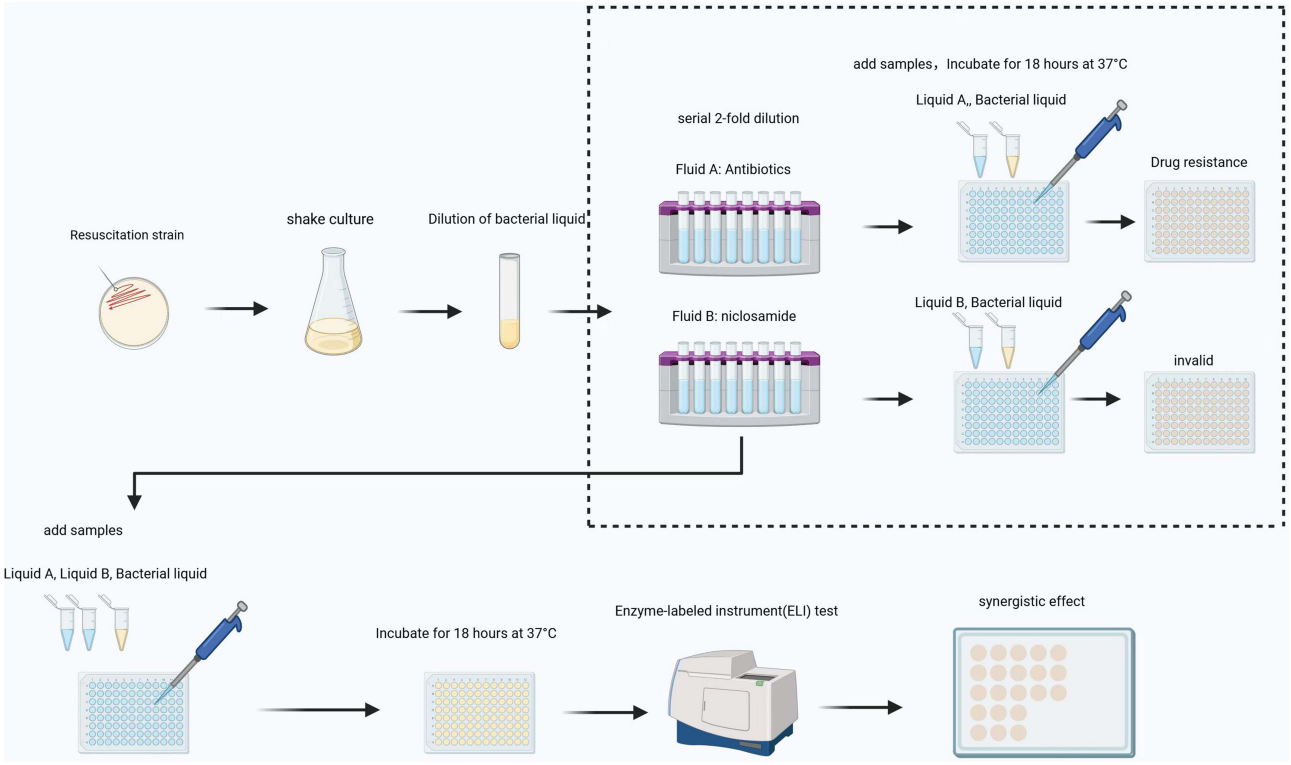

Figure 3 The experimental process of determining the synergistic effect of NIC and antibiotics by checkerboard method. Created in BioRender. Liu, Z. (2024) BioRender. com/m56s142.

mL NIC in combination with 0.25μg/mL colistin reduced the cell counts of the ATCC 17978 strain and the CECT 997 strain by 3.14 log CFU/mL and 4.62 log CFU/mL, respectively, compared to the 24-hour application of colistin alone. The combination of 0.65μg/mL NIC with 8μg/mL colistin and 0.65μg/mL NIC with 32μg/mL colistin showed higher synergistic activity against strain *A. baumannii* #11, *KPc*21, respectively. The synergistic effect was increased when the same concentration of colistin was added after 4 hours. Meanwhile, the efficacy of NIC in treating other Gram-negative bacteria (including *Acinetobacter, Enterobacteriaceae*, and *Pseudomonas*) when used in combination with colistin was observed, and the data showed that NIC could inhibit the development of colistin resistance.[100,102] The synergistic phenomenon was also verified in a mouse skin infection model.[102] Thus, NIC can reverse colistin resistance in Gram-negative bacteria and ameliorate the nephrotoxicity of colistin.[100] The above suggests that NIC, combined with colistin, may be a potential alternative treatment for colistin-resistant strains.

Pacios et al[103] found that NIC has synergistic activity against *K. pneumoniae* (including ST258, ST15-1, KPC-2, KPC-3, and K3325) when combined with Phe-Arg-β-naphthylamide dihydrochloride (PaβN). The results of the checker-board method assay showed that the MIC was reduced 64-fold and 250-fold, respectively, when NIC was combined with PaβN, and enhanced the intracellular concentration of NIC in *K. pneumoniae*. Both drugs showed low activity against *K. pneumoniae* when used alone (MIC $_{NIC}$ > 56.25μg /mL; MIC $_{PaβN}$: 500–1000μg/mL). Subsequent growth curve experiments showed that the synergistic effect was best when combined with 3.5μg/mL NIC and 4μg/mL PaβN. However, the combination produces an adaptive phenotype after continuous exposure, a feature that requires further investigation.

Berry et al[98] synthesized NIC-tobramycin compound 7 by copper-assisted azide-alkyne cycloaddition (CuAAC), which overcomes the problem of NIC's low water solubility. The checker-board method assay revealed that when 8μg/mL of compound 7 was combined with 4μg/mL of cefiderocol to treat *P. aeruginosa*, complete eradication of the bacteria was observed after 8 hours, and this combination reduced the cefiderocol MIC by 32-fold.

Niclosamide Derivative

Although NIC is a promising candidate for treating infections with MDR strains, it has significant limitations.[104] First, NIC has been reported to have good antimicrobial activity against Gram-positive bacteria such as *S. aureus* and *E. faecium*[51,59] but lower activity against Gram-negative bacteria.[100] Second, NIC's poor in vivo bioavailability and low cytotoxicity have limited its clinical application.[9] To further enhance the therapeutic efficacy of NIC against Gram-negative bacteria, improve its bioavailability, and reduce its cytotoxicity, new derivatives need to be synthesized.

Xu et al[105] synthesized a series of NIC New O-Alkylamino Binding Derivatives and evaluated the antibacterial activity of the new compounds against MDR bacteria. Among them, compounds 10 (Figure 4a) and 11 (Figure 4b) exhibit excellent antimicrobial activity. The MIC value of compound 10 for *KPC* ECT-997, *KPC*-28, and *E. coli NDM*-1 was 15μg/mL. At the same time, it showed excellent water solubility, and the HPLC analysis method determined the saturation concentration to 650 μg/mL. The MIC of compound 11 against the *KPC*-28 strain was 15μg/mL. Subsequent time-kill studies showed that the NIC derivatives, coupled with colistin, had varying degrees of synergistic effects on *Enterobacteriaceae* strains.

Lu et al[106] found that NIC enhanced the therapeutic effect of colistin on *P. aeruginosa* through high-throughput screening. Subsequently, a series of novel NIC-derived adjuvants were synthesized. The results of time-kill curves showed that 4μg/mL derivative 15 (Figure 4c) in combination with 1μg/mL colistin effectively reduced the activity of MDR *P. aeruginosa* DK2 (MIC_{COL}=256μg/mL) and inhibited the development of drug resistance. This effect was also demonstrated in other Gram-negative bacteria, including *A. baumannii* 186 (0.25μg/mL colistin + 8μg/mL15; 16-fold reduction in colistin MIC), *K. pneumoniae* 674 (1μg/mL colistin + 8μg/mL15; 256-fold reduction in colistin MIC), *E. cloacae* 15017 (0.5μg/mL colistin + 8μg/mL15; 16-fold re-duction in colistin MIC). Although NIC has low cytotoxicity, derivative 15 is not toxic in vivo even at high concentrations in mouse infection models. Thus, derivative 15 has a wider therapeutic window than NIC. Combining derivative 15 with colistin may be a promising therapy for MDR bacterial infections.

Berry et al[107] synthesized a series of novel derivatives of NIC, of which compound 4 (Figure 4d) reduced the MIC of *E. coli* 94393 against colistin from 8 μg/mL to 0.016 μg/mL at a concentration of 1.15 μg/mL. Compound 5a (Figure 4e)

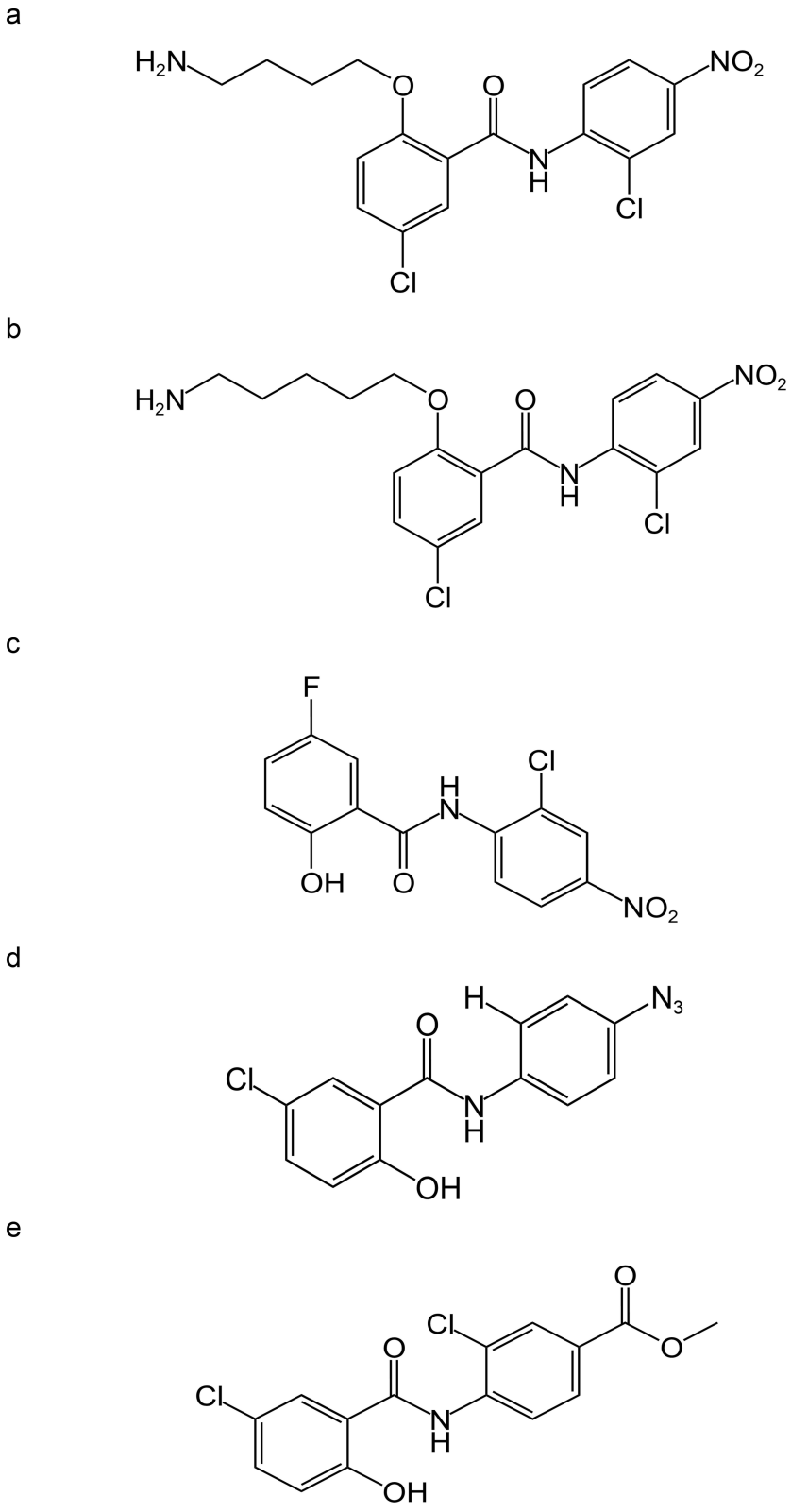

Figure 4 Structure of NIC derivatives: (**a**)compound 10 (HJC0431) with 4-aminobutyl moiety; (**b**) Compound 11 with 5-aminopentyl moiety; (**c**)Compound 15 containing 5-fluoro-2-methoxy- benzoic acid moiety; (**d**)Compound 4 with an azide moiety; (**e**)Compound 5a with methyl ester moiety.

was synthesized by replacing the nitro group in the chemical structure of NIC with methyl ester. It showed synergistic activity with colistin against selected bacterial strains, including Gram-negative bacteria such as *K. pneumoniae, A. baumannii, P. aeruginosa*, and *E. coli*. Eradication of colistin-resistant Gram-negative strains when 1μg/mL compound 5a was combined with 0.5μg/mL colistin. Subsequently, the toxicity of compound 5a was evaluated in two ovarian cancer cell lines, OVCAR-3 and COV362, and compared to NIC, which showed low cytotoxicity at high concentrations. In contrast, no cytotoxicity was detected for compound 5a. In conclusion, these results suggest that substituting the nitro group in NIC is a viable strategy for reducing cytotoxicity while maintaining synergy with colistin.

Mechanism of Action of Niclosamide

Suppress Quorum Sensing Systems

The quorum sensing system, which exists only in prokaryotes, directs the intercellular communication process between bacterial cells, modulating bacterial populations' behavior,[108] and is essential for bacterial survival and adaptation in the environment. This system interferes with the production of bacterial virulence factors,[109] increasing bacterial resistance to antibiotics, spreading disease, and making it difficult to treat bacterial infections in hospitals. The QS system has diffusible extracellular signals, mainly composed of Acyl-Homoserine Lactones (AHL), Autoinducing peptides (AIP), and Autoinducer-2 (AI-2), which produce specific signaling molecules to communicate with each other when an organism reaches its competence state.[108] Therefore, inhibition of the QS system may be a potential target for treating bacterial diseases.[110] The anthelmintic NIC was found to have a significant inhibitory effect on the QS response and the production of AHL signaling molecules in *P. aeruginosa*, inducing apoptosis by damaging cellular mitochondria or activating membrane surface death receptors.[74] N-(3-Oxododecanoyl)-L-homoserine lactone (3OC12-HSL) has been proposed to be the significant signal produced by *P. aeruginosa*.[111] The pathogenicity of *P. aeruginosa* depends on the expression of many virulence factors.[112] When the 3OC12-HSL signal molecule reaches a threshold concentration, it binds to *LasR* transcriptional regulators and triggers the expression of hundreds of genes.[111] NIC inhibits 3OC12-HSL production and 3OC12-HSL-dependent QS responses, as well as the production of several secreted virulence factors, such as pyocyanine, elastase, and rhamnolipid, which attenuates the pathogenicity of the galleria mellonella infection model's virulence.[74]

Inhibit Biofilm Formation

Biofilm is a highly organized and systematic microbial membrane polymer that adheres to the surface of a carrier and is coated with extracellular polymeric substances (EPS) and matrix mesh. The biofilm formation process typically includes the following steps: bacterial adhesion, colonization, secretion of extracellular polymers, biofilm maturation, and microbial detachment and recolonization.[113] Microbial communities within biofilms usually present a diverse population structure, and some of them may produce resistance genes and spread within the community through gene transfer and other means, resulting in bacterial antibiotic resistance.[114] The Two-Component Regulatory System (TCS) has been shown to regulate biofilm formation by inhibiting the autophosphorylation of histidine kinase components.[115] NIC has been shown to act on multiple TCSs of *S. aureus*, such as the LytSR and GraRS systems, which are essential in inhibiting bacterial growth, cell wall synthesis, biofilm formation, and antibiotic resistance.[52] Laser scanning confocal microscopy (LSCM) data confirmed that NIC inhibits the biological activity of *C. striatum* by degrading its biofilm matrix. Biofilm degradation exceeded 50% and 40% at drug concentrations of 1 × MIC and 1/2 × MIC, respectively, disrupting deeper biofilms and reducing the viability of matrix-resident bacterial cells.[63] NIC also acts on the *NDU1* gene of *C. albicans*, whose deletion prevents oxygen uptake by the bacteria, increases ROS accumulation, leads to mitochondrial depolarization, and prevents bacteria from forming biofilms.[87]

Affects Bacterial Cell Membrane Potential

Prokaryotes' respiration leads to the generation of a membrane potential (Δψ), which, together with the proton gradient (ΔpH) across the cytoplasmic membrane, forms the proton motive force (PMF).[116] In cells, Δψ is essential for physiological processes such as ATP synthesis, nutrient transport, and some biological motions (eg, flagellar rotation in bacteria).[117] Researchers Use LSCM to Assess Changes in the Fluorescence Intensity of the Fluorescent Probe DiSC3

(5) in the Cytoplasmic Membranes of Bacteria (increased fluorescence indicates membrane potential disruption and decreased fluorescence indicates dissipation of transmembrane pH). NIC was observed to effectively inhibit *C. difficile* growth by depolarizing the membrane potential and killing bacterial cells in logarithmic and stable phases in a concentration-dependent manner.[67] In addition, it can affect the physiological functions of *H. pylori* and *E. coli* by lowering the transmembrane pH and attenuating the generation of membrane potential.[80,102] Zeta potential measurements showed that NIC increased the negative surface charge of the bacterial outer membrane, thereby restoring colistin activity against Gram-negative bacteria.[101] Therefore, NIC can increase oxidative stress by depletion of bacterial PMF and decrease ATP production, which leads to cell death.[118]

Other Mechanisms

It has been observed that NIC can also exhibit antimicrobial effects through other mechanisms. NIC can disrupt the cell walls of *S. aureus* and *C. striatum*[54,63] and also inhibit bacterial growth by interfering with the bacterial catabolic pathway to reduce ATP levels in bacteria. In addition, NIC inhibits the production of α-HL, a key virulence factor of *S. aureus*, and attenuates pathogenicity in a mouse infection model.[54]

Discussion

The increase in AMR has made treating many common infectious diseases more difficult, and effective medicines are urgently needed to change this. The FDA approves NIC for the routine treatment of parasitic diseases, and it has recently shown potential for use in other infectious diseases. NIC showed potent activity against Gram-positive bacteria and fungi without drug resistance, and NIC significantly improved survival in the mouse infection model. In addition, NIC can serve as an antimicrobial surface coating for device-associated and hospital-acquired infections, clearing existing infections and preventing biofilm formation at very low concentrations. NIC's mechanism of action on bacteria includes affecting the quorum sensing system, biofilm formation, and bacterial cell membrane potential, thereby affecting bacterial growth.

Although NIC shows some potential for treating MDR infections, it still faces some challenges and limitations.

(1) Antibacterial range

The antibacterial effect of NIC on Gram-negative bacteria was relatively weak. In response to this phenomenon, colistin was combined with NIC to inhibit the exocytosis of Gram-negative bacteria to NIC. The entry of NIC into the bacterial cells affected the bacterial activity, and this phenomenon was also demonstrated in the combined effect of NIC with other drugs, such as PaβN. This suggests that although NIC is ineffective against Gram-negative bacteria, it effectively inhibits the growth of Gram-negative bacteria through synergistic effects. Future studies should further explore the synergistic effects of NIC with other antibiotics to develop more effective antimicrobial drug combinations and provide more options and opportunities for clinical treatment.

(2) Cytotoxicity

As a potential new antibacterial drug, the safety of NIC is the primary concern. NIC has a certain degree of cytotoxicity when used for an extended period. In addition to developing the derivatives and novel formulations mentioned in the text, researchers should thoroughly investigate their pharmacodynamic properties to reduce toxicity and enhance efficacy through topical or targeted delivery systems.

(3) Bioavailability

NIC is able to inhibit the activity of Gram-positive bacteria and fungi at very low concentrations and can be used as an adjuvant to enhance the activity of antibiotics against Gram-negative bacteria. However, low bioavailability limits its application. Therefore, researchers should aim to explore ways to improve the bioavailability of NIC, such as using

nanotechnology to microparticulate NIC to penetrate cell membranes more efficiently, thereby significantly enhancing its uptake and distribution in the body. Future research will focus on developing novel formulations of NICs aimed at their antimicrobial effects in a broader range of infectious diseases.

In conclusion, NIC shows excellent potential in treating infections caused by various microorganisms. This discovery brings new hope to the medical community and offers more options for treating patients. We hope that this review will provide our readers with valuable information and further promote the research and development of NIC in clinical applications.

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Disclosure

The authors report no conflicts of interest in this work.

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