Research Article

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Fabrication and characterization of lysine hydrochloride Cu(II) complexes and their potential for bombing bacterial resistance

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Abstract: The emergence of drug-resistant bacteria and the lack of effective antimicrobial agents have posed a threat to public safety, so a new efficient strategy is needed to deal with the increasing severity of bacteria. Herein, a substitute antibacterial agent with high stability and biocompatibility was synthesized by incorporating lysine hydrochloride and copper sulfate pentahydrate following the liquid-phase synthesis method. The composition and structure of the Cu(II) complex (Lys–Cu) were characterized by performing ultraviolet-visible spectroscopy, Fourier-transform infrared spectroscopy, X-ray diffraction (XRD), thermogravimetric analysis-differential scanning calorimetry, and single-crystal XRD. The crystal structure of Lys–Cu belongs to a monoclinic system, space group $P2_1$, with cell parameters of $a = 5.14350(10)$, $b = 16.8308(2)$, $c = 11.4915(2)$ Å, $Z = 2$, and $D = 1.548$ g cm$^{-3}$. Bacteriostatic tests were carried out on Escherichia coli and Staphylococcus aureus. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of Lys–Cu against S. aureus were 0.3125 and 0.9250 mg mL$^{-1}$, respectively. The MIC and MBC values of Lys–Cu against bacterial strain E. coli were 0.4685 and 0.9250 mg mL$^{-1}$, respectively.

Keywords: Lys–Cu, synthesis, characterization, biocompatibility, antimicrobial activity

1 Introduction

In recent years, with the development of economy and the acceleration of population movement, infectious diseases caused by bacterial infections have become a serious threat to global public health [1]. Antibiotics such as penicillin, streptomycin, tetracycline, and erythromycin can specifically target microbial infections, so they can effectively treat infectious diseases [2,3]. However, due to the abuse of antibiotics, bacteria have developed drug resistance, making antibiotics inactive, threatening the effective and sustainable treatment of infectious diseases [4]. If no action is taken to combat drug resistance, the global annual death rate is projected to reach 10 million by 2050 [5]. Therefore, it is an urgent need to develop antibacterial agents with high antibacterial efficiency and low bacterial resistance.

Many studies have found that metal-based antimicrobials can combine multiple mechanisms, such as by inducing oxidative stress, causing protein dysfunction, or destroying cell membranes to destroy and kill microbial cells; this poses a bigger challenge for resistant strains [6,7]. As a functional micronutrient, copper is required for important biological processes such as inhalation, iron transport, and normal cell metabolism [8]. In the early days, people used copper and its complexes to treat arthritis, lung gas, cholera, and tuberculosis [9].
Generally, the soluble copper salt, which can release free copper ions, is toxic, difficult to absorb, and has strong stimulation, which limits its application in clinical medicine [10]. Moreover, its toxicity can be reduced and its antibacterial property can be improved. For instance, by the formation of heterocyclic compound complexes with rare earth or transition-metal ions, the antibacterial effect of the rare-earth or metal complexes will be enhanced due to the synergistic effect [11]. Besides, the low biocompatibility of heterocyclic compounds limits the bacteria’s ability to absorb their metal complexes. In addition, the exact structure of organic ligands in copper complexes ultimately determines their primary use, while ligands containing nitrogen and oxygen donor systems may inhibit the production of enzymes and thus the growth of bacteria [12]. These complexes include amino acid complexes; because amino acids are biomolecules, their copper complexes should show good security. At the same time, the amino acid complexes of metal ions may be absorbed by cells in the form of small peptides, which can reduce the resistance of bacteria to heavy metals and increase the absorption rate [13].

Lysine is one of the essential amino acids for humans and mammals. The body cannot synthesize by itself and must be supplemented by food. L-lysine is a component of the active center of certain enzymes and has positive nutritional significance in promoting the growth and development of the human body and enhancing the body’s immunity [14]. Many studies have proved that L-lysine has therapeutic potential in preventing and managing various diseases [15]. Zhang et al. reported that L-lysine could ameliorate sepsis-induced acute lung injury by lipopolysaccharide-induced in mouse [16]. Shimomura et al. studied that L-lysine had a protective effect on arterial calcification in adenine-induced uremia rats [17]. Tian et al. reported that L-lysine had the anti-inflammatory effect on Microcystis aeruginosa [18]. As a ligand, lysine has three potential donor sites (two amino groups and one carboxyl group). Metal ions can form an inert complex by accepting the free electron pairs of O and N on amino acids. Brubaker and Busch and Duarte et al. have successfully synthesized the lysine copper complex and proved its chemical structure stability [19,20]. Abendrot et al. studied metal complexes of histidine, glycine, glutamic acid, methionine, tryptophan, and other amino acids that have shown good antibacterial properties [21], but there were few research studies on lysine metal complexes. Their mode of action may be the combination of amino acids and metal ions, which is likely to leave some potential free atom donors to enhance biological activity [22]. Therefore, as a potential antibacterial drug, lysine-chelated copper should be more efficient and safer [23].

The main purpose of this study is to synthesize an alternative antimicrobial agent with high antibacterial efficiency and biocompatibility. Herein, we report a safe and convenient method for the synthesis of a lysine–copper complex (Lys–Cu). The morphology, crystal structure, and properties of Lys–Cu were systematically characterized by directing ultraviolet and visible (UV-Vis) spectrophotometry, Fourier-transform infrared (FTIR) spectroscopy, X-ray diffractometry (XRD), thermogravimetric analysis-differential scanning calorimetry (TGA-DSC), and single-crystal XRD. Moreover, the antibacterial activity of Lys–Cu against Gram-positive bacteria Staphylococcus aureus and Gram-negative bacteria Escherichia coli was evaluated.

2 Experimental section

2.1 Materials

Copper sulfate pentahydrate (AR) was obtained from Shanghai Maclean Biochemical Technology Co., Ltd. Lysine hydrochloride (feed grade) was obtained from Guangzhou Tanke Biological Technology Co., Ltd. Luria Bertani (LB) broth (BR) was obtained from Aladdin Reagent (Shanghai) Co., Ltd. Agar (BR) was obtained from Hangzhou Best Biotechnology Co., Ltd. E. coli GDMCC 1.142 and S. aureus GDMCC 1.116 were provided by the Microbial Culture Collection Center of Guangdong Institute of Microbiology.

2.2 Compound synthesis

In this study, the synthesis of compounds referred to the method of Duarte et al. [19], and the synthesis process was optimized on the basis of this method. Feed-grade L-lysine hydrochloride (0.1 mol, 18.26 g) was added to CuSO₄·5H₂O (0.04 mol, 10.00 g); after mixing well, 25 mL of deionized water was added and stirred at 70°C for a few minutes until the solids were completely dissolved, and then 30% sodium hydroxide was used to adjust the pH to 7.5, and the reaction was continued for 30 min under these conditions. After the completion of the reaction, the solution was allowed to stand at room temperature for 12 h until the blue needle-like crystals were completely
precipitated. The product was filtered under reduced pressure and dried to constant weight.

2.3 Characterization

The KBr tablet method was used to perform infrared (FTIR) spectral characterization of the Cu(II) complex and lysine hydrochloride in the range of 500–4,000 cm⁻¹ using an FTIR spectrometer, with a scanning resolution of 4 cm⁻¹ and the number of scans of 36. Within the diffraction angle of 5–80°, XRD detection was performed on Cu(II) complexes and lysine hydrochloride by an X-ray powder diffractometer using Cu target Kα as the radiation source, with a wavelength (λ) of 0.154056 nm, tube pressure of 40 kV, tube flow of 15 mA, scanning speed of 5° min⁻¹. Under the protection of nitrogen, TG-DSC was performed with simultaneous thermal analysis to determine the thermal stability and melting temperature of the Cu(II) complex; the gas flow rate was 50 mL·min⁻¹, the heat range was 30–900°C, and the heating rate was 10°C·min⁻¹. The morphology of the sample was observed with a multifunctional optical microscope and an SEM.

2.4 Crystal structure determination

The complex single crystals with a size of 0.30 mm × 0.26 mm × 0.24 mm were selected, and the diffraction data were collected on a TGA5500 X-ray single-crystal diffractometer from Bruker, Germany. The data were collected at 150k, with MoKα ray (λ = 0.71073 Å) as the light source and the W/2θ scanning method. All diffraction data were corrected by the LP factor and empirical absorption, and the crystal structure was solved by the direct method; the data were refined by least squares and completed by the OLEX2 program. The hydrogen atom was found by the theoretical model, and from the difference Fourier diagram, the anisotropy was corrected.

2.5 Antimicrobial activity

The antibacterial activity of Cu(II) complexes against S. aureus and E. coli was determined by the transparent bacteriostatic circle method [24]. In all, 25 mL of LB broth solid medium was prepared in an Erlenmeyer flask, the mouth of the flask was sealed with aluminum foil, and the Erlenmeyer flask was placed in a high-pressure steam cooker for 20 min (0.1 MP, 121.5°C) for sterilization. In a sterile environment, the sterilized broth solid medium was poured into a sterile plate, cooled, and solidified. Then, the S. aureus and E. coli suspension was injected with a bacterial concentration of 1 × 10⁸ CFU·mL⁻¹ (100 μL) on different culture media, and coated evenly. At the center of the culture medium, a 6 mm round hole was made with a puncher, and the sample was added to the hole and cultured at 37°C for 24 h [25]. After three groups of experiments, the inhibition zone diameters (IZDs) were measured, and the average value was obtained.

2.6 Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC of the copper complex were determined using mini dilution [26]. First, frozen S. aureus and E. coli were resuscitated. S. aureus and E. coli were placed in LB broth under 37°C for 24 h. Then sterile LB broth was used to dilute the bacterial solution, so that the final inoculated bacterial density was about 1 × 10⁸ CFU·mL⁻¹. The copper complex solution with different concentrations was obtained by a double dilution method. The copper complex solution with different concentrations was obtained by the double dilution method. In ultra-clean Taichung, 100 μL of copper complex (different concentrations) and 100 μL of bacterial culture (1 × 10⁸ CFU·mL⁻¹) were added to a 96-well microtiter plate. The blank broth was used as a negative control, and the bacterial culture was used as a positive control. The 96-well microtiter plate was packed and incubated at 37°C for 24 h. The OD value at 600 nm was measured by an enzyme-labeled analyzer during the incubation period, and the growth curve of bacteria was obtained. The minimum sample concentration for inhibiting bacterial growth was the MIC, and the minimum drug concentration for killing 99.9% of the bacteria was the MBC. All the tests were performed on three parallel groups.

3 Results and discussion

The liquid-phase synthesis of Lys–Cu involves the optimization of the ratio of lysine hydrochloride to copper sulfate pentahydrate, time, temperature, and pH, as shown in Figure 1. Furthermore, the copper content of Lys–Cu
determined by an inductively coupled plasma (ICP) spectrometer was 13.80%, the lysine content determined by the Kjeldahl method was 75.95%, and the water content was 10.25%.

3.1 Morphological observation

As shown in Figure 2a and d, L-lysine hydrochloride was the white powder, but Lys–Cu crystals are blue, probably due to the introduction of copper ions. However, the texture of L-lysine hydrochloride was not as fine as that of Lys–Cu, which was mainly due to the difference in its microstructure and material composition [27]. To further analyze the microstructure, L-lysine hydrochloride and Lys–Cu were studied by an optical microscope and an SEM. As shown in Figure 2b and c, L-lysine hydrochloride was a yellow-white massive crystal with a rough crystal surface and many small pits, which may lead to the generation of many crystal defects and the deterioration of crystal quality [28,29]. The microstructure of Lys–Cu crystal was different from that of Lys, as shown in Figure 2e and f. Lys–Cu was the blue needle-like crystal with high crystallinity, good light transmittance, a smooth surface, and a series of small-size crystals attached around it. Macroscopic and microscopic morphology analyses showed that the copper complex sample was a new substance different from lysine.

3.2 Structural characterization

In order to further prove the successful chelation of the Lys–Cu chelate, UV-Vis spectroscopy and other analytical techniques were applied. For the UV-Vis spectra (Figure 3a), the maximum absorption peak of L-lysine hydrochloride is located at 202.00 nm, the maximum absorption peak of Lys–Cu is located at 236.00 nm, and a bathochromic shift of the Lys–Cu complexes is obviously observed. Due to the extension of the conjugated system of complexation, the formation of complexes will change the light absorption performance of the ligand, which can also prove the formation of Cu(II) complexes [30]. Furthermore, the FT-IR spectra of the complexes are shown in Figure 3b; compared with Lys, the key absorption peak of Lys–Cu has shifted significantly, and the intensity of the absorption peak has also changed. Lysine has a narrow and sharp characteristic absorption peak at 2,114 cm⁻¹ corresponding to the stretching vibration and bending vibration of the N–H bond [31]. In Figure 3b, this characteristic absorption peak disappeared, mainly because the Cu²⁺ in CuSO₄·5H₂O reacted with the amino group in lysine to form a

Figure 1: Scheme illustrating Lys–Cu preparation method and its application.
coordination bond. The band appearing at 592 cm$^{-1}$ is assigned to the contribution of the Cu–N symmetric vibration, indicating that α-N atoms participate in coordination. Another peak at 1,132 cm$^{-1}$ is attributed to C–N stretching, which is due to the contribution of share electrons of nitrogen atoms to copper atoms, leading to the increase of C–N bond bipolarity [32]. In addition, the peak at 1,586 cm$^{-1}$ in Lys–Cu complexes corresponds to the bending vibration of $-\text{NH}_2$ [33]. The absorption peaks ranging from 2,500 to 3,300 cm$^{-1}$ are ascribed to the $-\text{OH}$ vibrations of Lys, while for Lys–Cu, these characteristic peaks fall narrowly in the range of 2,900–3,390 cm$^{-1}$ and form several sharp small absorption peaks [34]. The presence of these characteristic peaks confirmed the formation of the Lys–Cu chelate.

Figure 3c shows the XRD spectra of l-lysine hydrochloride and Lys–Cu; the main peaks for l-lysine hydrochloride appear at $2\theta = 19.50^\circ$, 24.70$^\circ$, and 32.34$^\circ$. In comparison, the strong peaks for Lys–Cu appeared at $2\theta = 9.20^\circ$, 25.44$^\circ$, and 31.52$^\circ$. After lysine hydrochloride forms a complex with Cu(II), its main absorption peaks have been significantly shifted, and the relative strength has also changed, which reveals that lysine hydrochloride chelates with copper ions, rather than a simple mixture of lysine hydrochloride and copper sulfate. The XRD pattern of the lysine hydrochloride showed peak broadening, which indicates that the Cu(II) chelates have higher crystallinity than the starting material l-Lys. The stronger crystallinity of Cu(II) complexes may be due to the chelation of copper ions with amino and carboxyl groups in amino acids to form more stable pentacyclic compounds [35,36].

As depicted in Figure 3d, the thermal stability of lysine hydrochloride and Lys–Cu at 40–800°C was tested to evaluate the composition, thermal stability, and degradation of metal complexes. The TG of lysine hydrochloride revealed a step at 40–100°C with derivative thermogravimetry (DTG) peaks at 71.5°C because of the vaporization of the absorbed water. Another stage appeared between 250°C and 500°C due to the decomposition of lysine hydrochloride. In addition, the TG curve of Lys–Cu exhibited three stages corresponding to weight loss [37]. The first mass loss of Lys–Cu (7.54%) was related to the crystallization water loss. The secondly, Lys–Cu mass was loss about 60.45% in two identical organic forms [38]. Besides, the third stage in the range of 600–800°C due to the oxidation of copper was attributed to incomplete decomposition of COO–ligand fragments and the oxidation of copper. Interestingly, the residual amount of lysine hydrochloride (3.41%) was smaller than that of Lys–Cu (20.82%), further proving that the complex has good thermal stability.

In order to reveal the bonding mechanism of Lys–Cu and confirm its elemental composition and valence properties, XPS analysis was performed. The signals of C 1s, O 1s, N 1s, Cl 2p, and Cu 2p were found in the Lys–Cu curve, which indicated that Lys cooperated with Cu successfully. In the high-resolution Cu 2p spectrum (Figure 4b), the absorption peaks located at 951.78 and 931.88 eV are close to those of the typical Cu(II) X-ray photoelectron spectroscopy (XPS) peaks of Cu 2p$^{1/2}$ and Cu 2p$^{3/2}$, indicating that the Cu element is in the valence state of Cu$^{2+}$ [39]. The C 1s sub-peaks of Lys–Cu (Figure 4c) at 287.97, 284.69, and 284.11 eV correspond to C=O, C–O, and C–N.
bonds in the amino acid chain, respectively. In addition, the O 1s sub-peaks (Figure 4d) of Lys–Cu at 532.73 and 530.97 eV correspond to the C–O and C=O bonds in the carboxylic group, respectively [40]. Interestingly, the high-resolution N 1s spectra (Figure 4e) have two peaks: 399.2 eV for the nonprotonated nitrogen atom in the forms of amine or amide and 401.4 eV for the protonated nitrogen [41], indicating that during the coordination reaction, the more reactive end –NH₂ may protonate to –NH₃⁺. In the high-resolution Cl 2p spectra (Figure 4f), the sub peaks at 198.91 and 197.270 eV correspond to Cl 2p₁/₂ and Cl 2p₃/₂, respectively. It is reported that the binding energy of inorganic chlorine is less than 199 eV and that of organic chlorine is more than 200 eV [42]. Therefore, Cl in Lys–Cu may be involved in the coordination of Cu rather than C.

Figure 3: Structural characterization: (a) UV-Vis spectra, (b) FTIR spectra, (c) XRD patterns, and (d) TG-DTA curves.
3.3 X-ray crystal structure analysis

X-ray single-crystal diffraction analysis shows (Table 1) that the Lys–Cu chelate Cu[NH₃Cl(CH₂)₆CHNH₂COO]₂ · 2H₂O crystallizes in a monoclinic system and the structure has been solved in the P2₁ space group. Copper ions mostly form fourfold and sixfold coordination, but when the ligands are large, due to the repulsive force between the ligands, the copper ions tend to form a square-pyramidal or trigonal-bipyramidal geometry with fivefold coordination [43,44]. The coordinated geometry around Cu(II) is a distorted square-based pyramid configuration (Figure 5a), Cu(II) ions are located at the center of the base plane, which is defined by amino N and carboxylic O of the Lys molecule. The apical coordination site is occupied by a chlorine atom [45]. The distortion is caused by the Jahn–Teller effect of Cu(II) complexes [46,47]. In the crystals of the chelate, Cu(II) coordinates with the carboxyl oxygen (O₁, O₃) and the amino nitrogen (N₁, N₃) in the lysine ligand in the hydrochloride to form a two-membered ring, and the angle between the crystal surfaces of two pentagonal chelating rings is 20.263°.

![Figure 4: XPS spectra: (a) Cu in Lys–Cu, (b) Lys–Cu, (c) C in Lys–Cu, (d) O in Lys–Cu, (e) N in Lys–Cu, and (f) Cl in Lys–Cu.](image)

### Table 1: Crystallographic data for the compound

| Parameter          | Character          | Parameter          | Character          |
|--------------------|--------------------|--------------------|--------------------|
| Empirical formula  | C₁₂H₃₂Cl₂CuN₄O₆   | Index ranges       | -6 ≤ h ≤ 5, -21 ≤ k ≤ 21, -14 ≤ l ≤ 14 |
| Formula weight     | 462.85             | Temperature (K)    | 0.00(11)           |
| Crystal system     | monoclinic         | μ (mm⁻¹)           | 4.343              |
| Space group        | P2₁                | Volume (Å³)        | 993.02(3)          |
| a (Å)              | 5.14350(10)        | Z                  | 2                  |
| b (Å)              | 16.8308(2)         | F(000)             | 486                |
| c (Å)              | 11.4915(2)         | R (I > 2σ(I))      | R₁ = 0.1128, wR₁ = 0.2823 |
| α (°)              | 90                 | R (all data)       | R₂ = 0.1161, wR₂ = 0.2850 |
| β (°)              | 93.4340(10)        | Largest diff. peak and hole (e·nm⁻³) | 2.51/–1.99 |
| γ (°)              | 90                 |                    |                    |
| D (g·cm⁻³)         | 1.548              |                    |                    |
The geometry around Cu1 is distorted tetrahedral, with the angles varying from 83.9(3)° to 167.8(4)° (Table 2).

The aliphatic chains have an extended zig-zag conformation. The lysine molecules exist in the zwitterionic form with the carboxy protons transferred to the terminal –NH₂ groups [19]. This is because the amino acid terminal –NH₂ is active, and it is easy to combine with H⁺ to form –NH₃⁺ during the coordination reaction, which has a large steric hindrance and cannot coordinate with Cu(II). Due to the bridging character of Lys ligand with Cu(II), its carboxylic group presents a delocalized π system rather than a formal double bond: C1–O1 and C1–O2 distances are similar, 1.264 (11) and 1.254(12) Å, respectively [48]. The two independent lysine parts exhibit different molecular conformations, which are confirmed by the corresponding torsion angles of ∠N1–C2–C3–C4 = −72.217(2)° and ∠N3–C8–C9–C10 = −64.830(4)°.

Water molecules act as proton acceptors or proton donors, and their presence helps to improve the crystal packing and the formation of O–H...O hydrogen bonds, thus enhancing the stability of Cu(II) chelate [49]. There are obvious interactions between the molecules in the compound, mainly in the form of hydrogen bonds of N–H...O, N–H...Cl, N–H...Cl, and O–H...O (Table 3). These intermolecular hydrogen bonds between the water molecules and terminal –NH₃⁺ groups and the carboxyl oxygen and chlorine atoms make the intermolecular accumulation a three-dimensional structure (Figure 5b). The presence of this strong interaction will have a greater impact on the stability of Cu(II) [46]. The crystallographic data were deposited by the Cambridge Crystallographic Data Center, deposition number CCDC: 2039547.

### 3.4 Anti-bacterial activity analysis

To investigate the antibacterial activity of Lys–Cu against different strains, the antibacterial activity of Lys–Cu against the Gram-negative strain of *E. coli* and the Gram-positive strain of *S. aureus* was studied by the transparent bacteriostatic circle method. As shown in Figure 6a and b, obvious bacteriostatic circles appeared on agar plates, indicating that Lys–Cu had a bacteriostatic effect on both bacteria. The inhibition zone diameter of the drug for *E. coli* was 22.99 ± 0.1 mm and that for *S. aureus* was 24.61 ± 0.1 mm. In comparison, the inhibition effect of Lys–Cu on *S. aureus* was better than that on *E. coli*. Stāniā et al. studied the bacteriostatic activity of copper and cobalt amino acids complexes and found that similar results were obtained [50]. Similarly, aspartic acid complexes showed higher antibacterial activity against Gram-positive bacteria and lower antibacterial activity against...
Gram-negative bacteria [51]. Their differences in susceptibility to the test complex may be due to differences in the cell wall structure. The cell wall of Gram-positive bacteria is wider than that of Gram-negative bacteria, but without outer lipopolysaccharide, amino acid complexes are more likely to produce effects [52].

The growth curves of *S. aureus* and *E. coli* at different concentrations of copper lysine are shown in Figure 6e and f. High OD_{600} values indicate the higher concentration of bacteria and poorer antibacterial performance of Lys–Cu. In the control group (0 mg·mL⁻¹), the OD_{600} value of *E. coli* and *S. aureus* for 24 h was 0.65 and 0.73, respectively. After coculturing with Lys–Cu, the growth of both bacteria was inhibited, and the growth rate and the number of viable bacteria were lower than those of the control group. In addition, 100 μL of co-cultured solution for 24 h was added to the medium, evenly coated, and incubated at 37°C for 24 h to observe the growth of bacteria, as shown in Figure 6c and d. As can be seen, the higher the concentration of the drug, the fewer the bacteria in the culture medium. Interestingly, the inhibition effect of Lys–Cu on *S. aureus* was better than that on *E. coli* at the same concentration. Similarly, the same results can be seen in the bacterial growth curve; at the same drug concentration, the OD_{600} value of *S. aureus* is lower than that of *E. coli*. The above results are consistent with the experimental results of the inhibition zone diameter of Lys–Cu. On the other hand, the OD_{600} value of *S. aureus* treated with 0.3125 mg·mL⁻¹ Lys–Cu for 24 h was 0.32, the inhibition rate was 50.77%, and single colonies began to appear on the medium (Figure 6c2). Noteworthy, for *S. aureus* after treatment with 0.9250 mg·mL⁻¹ Lys–Cu for 24 h, the OD_{600} value was lower than the initial value (0 h), and no bacteria colonies were found on the culture medium (Figure 6c5), proving that all bacteria were killed. Thus, the MIC and MBC values of Lys–Cu against bacterial strain *S. aureus* were 0.3125 and 0.9250 mg·mL⁻¹, respectively. Meanwhile, the OD_{600} value of *E. coli* treated with 0.4685 mg·mL⁻¹ Lys–Cu for 24 h was 0.35, the inhibition rate was 52.05%, and single colonies began to appear on the medium (Figure 6d5). The killing effect of Lys–Cu on *E. coli* was more than 99.9% at 0.9250 mg·mL⁻¹ (Figure 6d3). In summary, the MIC and MBC values of Lys–Cu against bacterial strain *E. coli* were 0.4685 and 0.9250 mg·mL⁻¹, respectively.

Lys–Cu kills bacteria mainly by contact. When Lys–Cu contacts bacteria, Cu²⁺ will adsorb on the cell wall of bacteria, and the adsorbed Cu reacts with the peptidoglycan in the cell wall, resulting in cell collapse [53]. Simultaneously, copper ions interact with the negatively charged cell membrane, causing the bacteria to further decompose and die [54]. Copper lysine enters bacteria through endocytosis in the form of small peptides. The copper ions produced by the decomposition of copper lysine in bacteria interact with the mercapto group on the respiratory enzyme and inhibit the activity of the bacterial respiratory enzyme [55]. At the same time, Cu³⁺ combines with the bases of bacterial deoxyribonucleic acid (DNA) to form cross-links, which makes the bacterial nucleic acid solidify and achieve the purpose of antibacterial agent by hindering the reproduction of bacteria [56]. These effects of copper on bacteria may be enhanced by the presence of amino acids in copper chelates. On the other hand, reactive oxygen species produced by Lys–Cu can irreversibly destroy the cell membrane, DNA, and membrane proteins of bacteria, leading to bacterial cell death.

### Table 3: Distance and angles of hydrogen bonds in complex

| D–H...A     | d(D–H) (Å) | d(H...A) (Å) | d(D–A) (Å) | D–H...A (°) |
|-------------|------------|--------------|------------|------------|
| N(2)–H(2A)...O(6) | 0.890      | 1.900        | 2.778      | 168.81     |
| N(2)–H(2B)...Cl(2)¹ | 0.890      | 2.327        | 3.206      | 169.67     |
| N(2)–H(2C)...Cl(2)² | 0.890      | 2.320        | 3.185      | 164.03     |
| N(4)–H(4A)...Cl(1)³ | 0.890      | 2.255        | 3.133      | 169.07     |
| N(4)–H(4B)...Cl(5)⁴ | 0.890      | 2.088        | 2.848      | 142.67     |
| N(4)–H(4C)...O(2)⁵ | 0.890      | 1.911        | 2.795      | 171.64     |
| O(5)–H(5C)...Cl(2)⁶ | 0.850      | 2.400        | 3.239      | 169.59     |
| O(5)–H(5D)...O(2)⁷ | 0.850      | 1.973        | 2.817      | 172.39     |
| O(6)–H(6C)...Cl(1)⁸ | 0.850      | 2.356        | 3.200      | 171.98     |
| O(6)–H(6D)...O(3)⁹ | 0.850      | 2.303        | 3.001      | 139.59     |
| O(6)–H(6D)...O(4)¹⁰ | 0.850      | 2.223        | 3.006      | 153.19     |

Symmetry codes: 1 = –x+1, y+1/2, –z+1; 2 = –x, y+1/2, –z+1; 3 = –x+3, y–1/2, –z+2; 4 = x, y, z+1; 5 = x+1, y, z+1; 6 = x+1, y, z; 7 = x–1, y, z–1; 8 = x–1, y, z–1; 9 = x, y, z–1.
Figure 6: In vitro antimicrobial activity of: (a) Lys–Cu and S. aureus, (b) Lys–Cu and E. coli, (c) MIC and MBC of Lys–Cu against bacterial strain S. aureus, (d) MIC and MBC of Lys–Cu against bacterial strain E. coli, (e) OD600 growth of S. aureus after treatment of different concentrations of Lys–Cu, and (f) OD600 growth of E. coli after treatment of different concentrations of Lys–Cu.

Figure 7: In vitro antimicrobial activity of Lys–Cu via reactive oxygen species.
death [57]. Figure 7 shows the antibacterial mechanism of Lys–Cu.

4 Conclusion

In this study, Lys–Cu was successfully synthesized by a safe and simple method. The results of morphological observation show that Lys–Cu is a blue needle-like crystal. The synthesis of the complex was confirmed by UV-Vis spectra, the presence of functional groups was confirmed by FT-IR spectra and the high crystallinity of Lys–Cu was confirmed by XRD spectra; the results of thermogravimetric analysis show that it has good thermal stability. In addition, XPS analysis reveals the bonding mechanism of Lys–Cu and confirms its elemental composition and valence state properties. Single-crystal XRD analysis revealed that lysine and copper M:L stoichiometric ratio is 2:1, containing two molecules of crystal water, and the structural formula is Cu[ND2Cl(CH2)nC1H2N2COO]2·2H2O. The complex molecules of Lys–Cu are organized into a three-dimensional hydrogen-bonding network. Antibacterial analysis found that Lys–Cu showed promising antibacterial properties, and its antibacterial properties against Gram-positive bacteria were superior to those against Gram-negative bacteria. In summary, Lys–Cu is a potential alternative antibacterial agent with high stability, high antibacterial efficiency, and good biocompatibility.

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