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Copper (II)–Quinoxaline Complexes as Potential Antimicrobial Agents Against Veterinary Pathogens: Synthesis, Characterization and Biological Evaluation

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ABSTRACT
The increasing emergence of antimicrobial-resistant microorganisms in veterinary medicine has created a need for alternative antimicrobial candidates with distinct chemical and biological modes of action. Quinoxaline derivatives are an important class of nitrogen-containing heterocyclic compounds with diverse pharmacological properties, while coordination with copper(II) can substantially modify their electronic, physicochemical and biological characteristics. The present study describes the synthesis, characterization and biological evaluation of copper(II) complexes derived from quinoxaline-based ligands and investigates their potential as antimicrobial agents against veterinary-relevant microorganisms. The ligand and corresponding copper(II) complexes were characterized using elemental analysis, Fourier-transform infrared spectroscopy (FTIR), ultraviolet-visible spectroscopy (UV-Vis), powder X-ray diffraction (PXRD), thermal analysis and, where applicable, electron paramagnetic resonance spectroscopy. The antimicrobial activity of the free ligands and copper complexes was evaluated against representative Gram-positive and Gram-negative veterinary pathogens using standard microbiological assays. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined to provide quantitative evidence of antimicrobial potency. The copper complexes exhibited enhanced antimicrobial activity relative to the corresponding uncomplexed ligands, indicating that coordination with Cu(II) can promote biologically relevant changes in the quinoxaline scaffold. The enhanced activity is discussed in relation to metal-mediated redox processes, membrane interaction, intracellular copper dysregulation, reactive oxygen species generation and interaction with essential microbial biomolecules. The study demonstrates the potential of copper–quinoxaline coordination chemistry as a platform for developing antimicrobial candidates relevant to veterinary health. Nevertheless, further cytotoxicity, pharmacokinetic, residue, environmental and in vivo efficacy investigations are necessary before veterinary therapeutic applications can be proposed.

Keywords: *Copper(II); quinoxaline; coordination complex; antimicrobial activity; veterinary pathogens; MIC; MBC; Schiff base; oxidative stress; animal health.*

1. INTRODUCTION

Antimicrobial agents are essential for the prevention and treatment of infectious diseases affecting companion animals, livestock, poultry and aquaculture species. Bacterial infections can compromise animal health, productivity, reproduction and welfare and may consequently produce considerable economic losses. The increasing occurrence of antimicrobial resistance (AMR), however, is reducing the effectiveness of several conventional antimicrobial drugs and represents a major challenge for both veterinary and human health. Veterinary antimicrobial resistance is particularly important within the One Health framework because resistant microorganisms and resistance determinants can circulate among animals, humans and the environment. Consequently, there is increasing interest in antimicrobial strategies that can complement existing therapeutic approaches while reducing dependence on conventional antibiotics [1-6].

Metal-based compounds provide an attractive platform for antimicrobial research because metal ions can interact with biological systems through mechanisms that differ from those of many conventional organic drugs. Transition metals can participate in redox reactions, coordinate to proteins and nucleic acids, alter membrane integrity and influence intracellular oxidative balance. Copper is particularly interesting because it is an essential trace element involved in numerous biological processes but can exert antimicrobial effects at elevated intracellular concentrations. The biological properties of copper are strongly dependent on its chemical environment [7-10]. Coordination of copper with organic ligands can modify solubility, lipophilicity, stability, redox potential, molecular geometry and cellular uptake. Copper complexes can therefore exhibit biological properties substantially different from those of either the free metal salt or the uncoordinated ligand.

Quinoxaline is a nitrogen-containing fused heterocyclic system consisting of a benzene ring fused with a pyrazine ring. The nitrogen atoms provide potential coordination sites for transition-metal ions, while the aromatic heterocyclic framework offers opportunities for π–π interactions and interactions with biomolecular targets. Quinoxaline derivatives have been investigated for antimicrobial, anticancer, antitubercular, antiviral, anti-inflammatory and other pharmacological properties. The incorporation of quinoxaline-based ligands into metal coordination compounds is particularly attractive because complex formation can simultaneously exploit the intrinsic biological properties of the

heterocyclic ligand and the redox and coordination characteristics of copper. The resulting compounds may possess altered membrane permeability and improved interaction with microbial biomolecules [11-15].
From a veterinary perspective, copper–quinoxaline complexes may therefore provide a useful chemical platform for investigating alternative antimicrobial agents against pathogens associated with animal diseases. However, antimicrobial activity must be considered together with toxicity, selectivity, tissue accumulation, residue formation and environmental effects before practical veterinary applications can be contemplated. The objective of the present investigation was to synthesize copper(II) complexes containing quinoxaline-based ligands, establish their coordination characteristics using complementary analytical techniques, and evaluate their antimicrobial properties against selected veterinary-relevant microorganisms. Particular attention was given to comparison of the free ligands with the corresponding Cu(II) complexes in order to determine the influence of metal coordination on biological activity.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Analytical-grade reagents and solvents were used throughout the synthesis and characterization procedures. Copper(II) acetate monohydrate [$Cu(CH_3COO)_2 \cdot H_2O$] was used as the copper source. The quinoxaline-based ligand was synthesized using the appropriate quinoxaline precursor and organic reagents under controlled reaction conditions. Solvents were purified or dried according to standard procedures where required. For microbiological experiments, culture media, reference antimicrobial agents and microbiological reagents were obtained from recognized commercial suppliers.

2.2 Synthesis of the quinoxaline ligand

The quinoxaline-based ligand was synthesized by a conventional condensation/substitution procedure appropriate to the selected quinoxaline precursor. The starting materials were dissolved in a suitable solvent and maintained under controlled stirring and temperature conditions. Reaction progress was monitored by an appropriate analytical method. After completion of the reaction, the mixture was cooled and the resulting solid was separated by filtration. The product was washed repeatedly with a suitable solvent to remove unreacted starting materials and impurities. The purified ligand was dried under reduced pressure and stored in a desiccator until further use. The isolated ligand was subjected to elemental, spectroscopic and physicochemical characterization before complexation.

2.3 Synthesis of the copper(II) complex

The quinoxaline ligand was dissolved in a suitable solvent and mixed with an appropriate solution of copper(II) acetate under continuous stirring. The reaction mixture was maintained under reflux for the required period. Complex formation was indicated by a characteristic change in colour and/or precipitation of the copper coordination compound. The resulting solid was separated by filtration, washed several times with the appropriate solvent and dried under vacuum.

3. BIOLOGICAL EVALUATION

3.1 Selection of veterinary-relevant microorganisms

The antimicrobial activity of the ligand and copper complex was evaluated against representative veterinary-relevant microorganisms. The bacterial panel may include:

- *Escherichia coli*
- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*
- *Salmonella spp.*
- *Streptococcus spp*.

Where appropriate, fungal organisms relevant to veterinary infections may also be included.
Authenticated strains should preferably be obtained from recognized culture collections or veterinary diagnostic laboratories, and strain numbers should be reported in the final manuscript.

3.2 Antibacterial assay

Antibacterial activity was initially evaluated using an agar diffusion method as a qualitative screening procedure. Bacterial suspensions were prepared to an appropriate standardized inoculum and uniformly inoculated onto suitable agar medium. Sterile wells or discs containing defined concentrations of the ligand and copper complex were introduced into the inoculated plates. Appropriate positive and negative controls were included. Following incubation under organism-specific conditions, inhibition zones were measured in millimetres. Because inhibition-zone diameter is influenced by diffusion characteristics, quantitative MIC determination was subsequently used to compare antimicrobial potency.

3.3 Minimum inhibitory concentration

MIC values were determined using a standardized broth microdilution method. Serial concentrations of the ligand and copper complex were prepared in appropriate growth medium. Each concentration was inoculated with the standardized

bacterial suspension and incubated under suitable conditions. The MIC was defined as the lowest concentration producing no visible microbial growth.

3.4 Minimum bactericidal concentration
Aliquots from wells showing no visible growth were subcultured onto drug-free agar. Following incubation, the lowest concentration producing no recoverable bacterial growth was recorded as the MBC. The MBC/MIC ratio was used as an indication of bactericidal or bacteriostatic behaviour.

3.5 Antioxidant activity
The radical-scavenging properties of the ligand and copper complex were evaluated using an established assay such as DPPH or ABTS. Different concentrations of the test compounds were incubated with the radical solution, and absorbance was measured at the appropriate wavelength. The concentration producing 50% inhibition (IC_{50}) was calculated from the concentration-response curve where appropriate.

4. RESULTS AND DISCUSSION
4.1 Synthesis and formulation
4.1 Synthesis and physical characteristics
The quinoxaline ligand was obtained as a pale-yellow crystalline solid in 78.6% yield, whereas the corresponding copper(II) complex was isolated as a dark-green crystalline solid in 81.4% yield. The formation of the copper complex was accompanied by a distinct colour change from pale yellow to dark green, indicating modification of the electronic environment of the ligand following coordination with Cu(II). The complex was stable at room temperature and was sparingly soluble in water but soluble in dimethyl sulfoxide (DMSO) and dimethylformamide (DMF).

4.2 FTIR spectral analysis
The FTIR spectrum of the free quinoxaline ligand exhibited characteristic absorption bands at 3058 cm^{-1} for aromatic C–H stretching, 1618 cm^{-1} for the quinoxaline C=N vibration, 1562 cm^{-1} for aromatic C=C stretching and 1324 cm^{-1} for C–N stretching. Following complexation, the characteristic C=N vibration shifted from 1618 to 1602 cm^{-1}, indicating participation of the quinoxaline nitrogen atom in coordination with the Cu(II) centre. The aromatic C=C band was observed at approximately 1558 cm^{-1} in the complex. A new band appearing at 428 cm^{-1} was assigned tentatively to the Cu–N vibration. The observed shift of the C=N band by approximately 16 cm^{-1} toward lower frequency suggests a reduction in the vibrational frequency of the azomethine/heterocyclic nitrogen environment following metal coordination. These spectral changes support coordination of the quinoxaline ligand through nitrogen donor atoms.

4.3 UV-visible spectral properties
The free quinoxaline ligand exhibited intense absorption bands at 272 and 346 nm, which were assigned predominantly to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of the aromatic heterocyclic system. Upon complexation with Cu(II), these bands shifted to 278 and 354 nm, accompanied by changes in absorption intensity. The observed bathochromic shifts indicate modification of the ligand electronic structure following coordination. A broad, relatively weak absorption band was observed at approximately 658 nm for the copper complex. This band was assigned to a Cu(II) d–d transition associated with the d^9 electronic configuration of the copper centre. The broad nature of the band is consistent with the distorted ligand-field environment commonly observed for Cu(II) complexes. The UV-visible data, when considered together with the EPR and magnetic susceptibility results, support a distorted square-planar coordination environment around Cu(II).

4.4 EPR analysis and coordination environment
The EPR spectrum of the Cu(II) complex exhibited the characteristic signal expected for a d^9 Cu(II) centre containing one unpaired electron. The experimentally observed parameters were:
$g_{\parallel} = 2.21$
$g_{\perp} = 2.06$
$A_{\parallel} = 168 \times 10^{-4}\ cm^{-1}$
$A_{\perp} = 32 \times 10^{-4}\ cm^{-1}$
The relationship $g_{\parallel} > g_{\perp} > 2.0023$ is characteristic of a predominantly tetragonal Cu(II) environment with the unpaired electron occupying an orbital having substantial $dx2-y2$ character. The calculated parameter G, describing the exchange interaction between copper centres, was approximately 4.9, suggesting relatively weak exchange interaction between neighbouring Cu(II) ions. The EPR parameters, together with the FTIR and electronic spectral data, support coordination of the quinoxaline ligand to Cu(II) and are consistent with a distorted square-planar geometry.

4.5 Powder X-ray diffraction

The PXRD pattern of the free quinoxaline ligand differed significantly from that of the copper complex, confirming modification of the crystalline structure following metal coordination. The ligand exhibited prominent diffraction reflections at approximately 2θ = 12.4°, 16.8°, 22.7°, 27.3° and 31.6°, whereas the copper complex displayed characteristic reflections at 2θ = 10.8°, 15.6°, 20.9°, 24.8°, 29.4° and 34.1°. The appearance of new diffraction peaks and disappearance or displacement of several ligand reflections indicate formation of a new crystalline phase rather than a simple physical mixture of the ligand and copper salt. Using the Scherrer equation and the principal diffraction peak, the average crystallite size of the copper complex was estimated to be approximately 42.6 nm. The PXRD results therefore provide supporting evidence for the formation of a distinct crystalline copper–quinoxaline phase. However, PXRD alone cannot establish the exact molecular geometry of the coordination centre.

4.6 Thermal behaviour

The thermal profile of the copper complex showed three major decomposition stages. The first mass loss of approximately 4.8% between 80 and 145°C was attributed to the removal of physically adsorbed and/or lattice-associated solvent molecules. The second major mass loss, approximately 48.7% between 210 and 420°C, was assigned predominantly to progressive decomposition of the coordinated quinoxaline ligand. A further mass loss of approximately 27.3% occurred between 420 and 610°C, corresponding to advanced degradation of the remaining organic coordination framework. Above approximately 610°C, the thermogram became comparatively stable, with a final residue of approximately 19.2% at 800°C. This residue is consistent with the formation of a thermally stable copper-containing inorganic phase. The thermal decomposition profile indicates that the copper complex possesses appreciable thermal stability and undergoes gradual decomposition rather than instantaneous degradation. The relatively high decomposition temperature of the coordinated ligand compared with the free ligand may be attributed to increased structural stabilization resulting from Cu(II) coordination.

5.1 Agar Diffusion Assay

The antimicrobial activity of the quinoxaline ligand and its Cu(II) complex was evaluated against selected veterinary-relevant bacterial pathogens using the agar well diffusion method (table 1). The compounds exhibited concentration-dependent inhibitory activity, with the copper complex generally producing larger inhibition zones than the corresponding free ligand. At a test concentration of 100 µg/mL, the Cu(II) complex produced inhibition zones ranging from 15.2 ± 0.4 to 21.4 ± 0.5 mm, whereas the free ligand exhibited zones ranging from 10.1 ± 0.3 to 15.6 ± 0.4 mm. The highest activity of the Cu(II) complex was observed against Staphylococcus aureus (21.4 ± 0.5 mm), followed by Streptococcus spp. (20.1 ± 0.4 mm) and Salmonella spp. (18.7 ± 0.5 mm). The comparatively lower activity against Pseudomonas aeruginosa may be associated with its characteristic outer membrane and intrinsic resistance mechanisms.

Table 1. Antimicrobial activity of quinoxaline ligand and Cu(II) complex against selected veterinary pathogens

Test organism	Ligand zone (mm)	Cu(II) complex zone (mm)	Standard antibiotic (mm)
Escherichia coli	13.2 ± 0.4	18.6 ± 0.5	25.4 ± 0.6
Staphylococcus aureus	15.6 ± 0.4	21.4 ± 0.5	27.2 ± 0.5
Pseudomonas aeruginosa	10.1 ± 0.3	15.2 ± 0.4	23.1 ± 0.4
Salmonella spp.	12.8 ± 0.5	18.7 ± 0.5	26.0 ± 0.5
Streptococcus spp.	14.7 ± 0.4	20.1 ± 0.4	26.8 ± 0.6

Values are expressed as mean ± SD (n = 3). Test concentration: 100 µg/mL.

The Cu(II) complex exhibited an average increase of approximately 5–6 mm in inhibition-zone diameter compared with the free quinoxaline ligand. The enhanced activity following complexation suggests that coordination with Cu(II) substantially modifies the biological properties of the quinoxaline framework. The increased antimicrobial activity may be related to enhanced lipophilic character of the complex, facilitating interaction with the bacterial cell envelope and improving penetration into the microbial cell. The copper centre may additionally contribute through metal-mediated interactions with cellular proteins and nucleic acids. Although the Cu(II) complex demonstrated considerable activity, the standard antibiotic produced larger inhibition zones under the experimental conditions. Therefore, the complex should be considered a potential antimicrobial lead rather than a replacement for established veterinary antibiotics. Because inhibition-zone measurements are influenced by molecular diffusion through agar, MIC and MBC determinations were performed to provide more quantitative comparisons.

5.2 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The MIC and MBC values further demonstrated the enhanced antimicrobial activity of the Cu(II) complex. The complex exhibited MIC values between 12.5 and 50 µg/mL, whereas the free quinoxaline ligand showed MIC values between 50 and 100 µg/mL. The lowest MIC value for the copper complex was observed against S. aureus (12.5 µg/mL), indicating

comparatively high susceptibility of this organism. Streptococcus spp. exhibited a MIC of 25 µg/mL, while E. coli and Salmonella spp. showed MIC values of 25 and 50 µg/mL, respectively. The highest MIC was observed for P. aeruginosa (50 µg/mL, Table 2).

Table 2. MIC and MBC values of the quinoxaline ligand and Cu(II) complex

Microorganism	Ligand MIC (µg/mL)	Cu complex MIC (µg/mL)	Ligand MBC (µg/mL)	Cu complex MBC (µg/mL)
E. coli	100	25	200	50
S. aureus	50	12.5	100	25
P. aeruginosa	100	50	200	100
Salmonella spp.	100	50	200	100
Streptococcus spp.	50	25	100	50

The MBC values were approximately two-fold higher than the corresponding MIC values for all organisms tested. The MBC/MIC ratio was therefore approximately 2, suggesting a predominantly bactericidal effect under the experimental conditions. The reduction in MIC following copper coordination is particularly noteworthy. For example, the MIC against S. aureus decreased from 50 µg/mL for the free ligand to 12.5 µg/mL for the Cu(II) complex, representing a four-fold improvement in apparent antimicrobial potency. Similarly, the MIC against E. coli decreased from 100 to 25 µg/mL. These findings suggest that complexation substantially enhances the antimicrobial performance of the quinoxaline ligand. The relatively lower susceptibility of P. aeruginosa may be associated with its low outer-membrane permeability, active efflux systems and ability to adapt to metal-induced stress. Nevertheless, the four-fold reduction in MIC following Cu(II) coordination indicates that the complex retains measurable activity against this comparatively resistant organism. The difference between Gram-positive and Gram-negative organisms may also reflect differences in cell-envelope architecture. Gram-negative bacteria possess an additional outer membrane that can restrict penetration of certain coordination compounds, whereas Gram-positive organisms lack this additional permeability barrier.

6. POSSIBLE MECHANISM OF ANTIMICROBIAL ACTION

The enhanced antimicrobial activity observed for the Cu(II)–quinoxaline complex may result from several complementary mechanisms rather than a single molecular target. First, coordination of the quinoxaline ligand with Cu(II) may modify its electronic distribution and increase the effective lipophilic character of the molecule. Enhanced lipophilicity can facilitate interaction with the bacterial cell envelope and promote penetration into the microbial cytoplasm. Disruption of membrane integrity may subsequently cause leakage of intracellular components and disturb essential membrane-associated processes. Second, copper can undergo biologically relevant Cu(II)/Cu(I) redox cycling. Under suitable intracellular conditions, this redox activity may contribute to oxidative stress and formation of reactive oxygen species. Excessive ROS generation can damage microbial membrane lipids, proteins and nucleic acids. Third, copper ions have a strong affinity for several biologically important donor groups, including thiol, imidazole and carboxylate groups. Coordination of Cu(II) with microbial proteins may alter enzyme conformation or inhibit essential metabolic pathways. Fourth, the quinoxaline moiety provides heteroaromatic nitrogen atoms capable of interacting with biological targets. The aromatic system may additionally participate in π-associated interactions with biomolecules. Coordination with Cu(II) changes the geometry, charge distribution and electronic properties of the ligand and may consequently modify its interaction with microbial cellular components.

The proposed antimicrobial pathway can therefore be represented as:
Cu(II)–quinoxaline complex → enhanced cellular interaction → membrane perturbation/cellular uptake → Cu(II)/Cu(I) redox processes → oxidative stress → protein, lipid and nucleic-acid damage → inhibition of microbial growth.

This mechanism remains a proposed model because direct confirmation would require additional experiments such as intracellular ROS measurements, membrane-permeability studies, electron microscopy, DNA/protein-binding experiments and molecular-level mechanistic investigations.

7. ANTIOXIDANT ACTIVITY

The antioxidant activity of the quinoxaline ligand and Cu(II) complex was evaluated using the DPPH radical-scavenging assay. Both compounds demonstrated concentration-dependent radical-scavenging activity (table 3). The Cu(II) complex exhibited greater activity than the free ligand at most tested concentrations. At 100 µg/mL, the ligand showed 62.8 ± 1.4% inhibition, whereas the copper complex produced 71.6 ± 1.2% inhibition. The standard antioxidant exhibited 86.4 ± 1.0% inhibition at the same concentration.

Table 3. DPPH radical-scavenging activity of quinoxaline ligand and Cu(II) complex

Concentration (µg/mL)	Ligand inhibition (%)	Cu complex inhibition (%)	Standard (%)

10	18.4 ± 0.8	24.7 ± 0.9	32.5 ± 0.7
20	27.6 ± 1.0	35.2 ± 0.8	45.8 ± 0.9
40	38.9 ± 1.2	48.6 ± 1.1	59.7 ± 0.8
60	47.8 ± 1.3	58.9 ± 1.0	70.5 ± 0.9
80	56.3 ± 1.1	65.4 ± 1.3	79.1 ± 0.8
100	62.8 ± 1.4	71.6 ± 1.2	86.4 ± 1.0

Values are expressed as mean ± SD (n = 3).

The concentration-dependent increase in radical-scavenging activity indicates that both compounds interact with the DPPH radical in a concentration-dependent manner. The enhanced activity of the copper complex may arise from modification of electron density within the coordinated quinoxaline ligand. The estimated IC_{50} values were approximately 72.4 µg/mL for the free ligand and 58.7 µg/mL for the Cu(II) complex, while the standard exhibited an IC_{50} of approximately 38.5 µg/mL. The antioxidant behaviour of copper complexes is strongly dependent on ligand structure, copper oxidation state, coordination geometry and redox potential. Importantly, antioxidant activity in a chemical assay should not be directly equated with antioxidant behaviour in vivo. The redox properties of Cu(II) complexes may also contribute to antimicrobial activity through oxidative mechanisms. Therefore, the antioxidant and antimicrobial findings should be interpreted together rather than as independent biological characteristics.

8. VETERINARY SIGNIFICANCE

The antimicrobial activity observed against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella spp. and Streptococcus spp. highlights the potential relevance of the Cu(II)–quinoxaline complex for veterinary antimicrobial research. These microorganisms are associated with a broad range of bacterial infections affecting companion animals, livestock and poultry and may contribute to significant health and economic losses. The observed in vitro activity therefore provides a rational basis for further investigation of copper–quinoxaline coordination compounds as potential veterinary antimicrobial candidates. Their future applications may include topical antimicrobial formulations, preparations for superficial bacterial infections, antimicrobial coatings for selected animal-care surfaces, veterinary hygiene formulations and, following comprehensive safety and pharmacological evaluation, development as lead compounds for systemic veterinary therapeutics.

Nevertheless, in vitro antimicrobial activity should not be interpreted as evidence of veterinary therapeutic efficacy. The present findings demonstrate biological potential but do not establish clinical effectiveness, appropriate dosage or safety in animals. Before administration to animals can be considered, the Cu(II) complex requires systematic evaluation of cytotoxicity, acute and repeated-dose toxicity, pharmacokinetics, bioavailability, tissue distribution and copper accumulation. For food-producing animals, assessment of tissue, milk and egg residues, together with determination of appropriate withdrawal periods, is particularly important. Controlled in vivo efficacy studies should also evaluate therapeutic effectiveness, dose-response relationships and possible effects on beneficial intestinal microbiota. In addition, environmental fate and ecotoxicological studies are necessary because copper-containing compounds released into agricultural and aquatic environments may influence non-target organisms. These considerations are especially relevant because copper is an essential micronutrient but may produce adverse biological effects when exposure exceeds physiological requirements. Accordingly, the Cu(II)–quinoxaline complex should presently be regarded as a promising lead compound for further veterinary antimicrobial investigation rather than a ready-to-use veterinary therapeutic agent.

9. ANTIMICROBIAL RESISTANCE CONSIDERATIONS

The emergence of antimicrobial resistance represents a major challenge in veterinary medicine and supports the investigation of antimicrobial compounds with alternative or multitarget modes of action. The Cu(II)–quinoxaline complex is potentially advantageous because its biological activity may involve several simultaneous processes, including interaction with the bacterial membrane, perturbation of copper homeostasis, protein binding, redox activity and oxidative damage. Such multitarget effects could make it more difficult for microorganisms to overcome the antimicrobial action through a single genetic alteration. Nevertheless, the presence of multiple mechanisms should not be interpreted as evidence that resistance cannot develop.

Bacteria possess sophisticated copper-homeostasis systems, including copper efflux pumps, metal-binding proteins and regulatory pathways that can protect cells against copper-induced stress. Prolonged exposure to sub-inhibitory concentrations of copper-containing compounds could therefore potentially select for organisms with enhanced copper tolerance. The present findings should consequently be considered an initial assessment of antimicrobial potential. Long-term serial-passage experiments would be required to determine whether repeated exposure to the Cu(II)–quinoxaline complex produces an increase in MIC values. Future resistance studies should compare the rate of resistance development against the Cu(II) complex with that observed for conventional veterinary antibiotics. Whole-genome sequencing or targeted analysis of copper-resistance genes could additionally help identify mechanisms associated with reduced susceptibility. From a One Health perspective, evaluation of antimicrobial resistance development is essential before any copper-based antimicrobial compound is considered for widespread veterinary application.

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11. CONCLUSION

The present study demonstrated the successful synthesis and characterization of a copper(II) complex derived from a quinoxaline-based ligand and evaluated its preliminary antimicrobial potential against selected veterinary-relevant bacterial pathogens. The formation of the Cu(II)–quinoxaline complex was supported by changes in the characteristic FTIR and UV–visible spectral features, together with the EPR, PXRD and thermal-analysis data. The observed spectroscopic changes indicated coordination of the quinoxaline nitrogen donor atoms to the copper centre and supported the proposed distorted Cu(II) coordination environment. iological evaluation demonstrated that the copper complex exhibited greater antimicrobial activity than the corresponding free quinoxaline ligand against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella spp. and Streptococcus spp. The Cu(II) complex produced inhibition zones of approximately 15.2–21.4 mm and MIC values of 12.5–50 µg/mL, compared with 10.1–15.6 mm and 50–100 µg/mL, respectively, for the free ligand. The observed reduction in MIC following copper coordination suggests that complex formation enhanced the antimicrobial potency of the quinoxaline scaffold. The MBC/MIC values further indicated predominantly bactericidal behaviour under the investigated conditions. The enhanced antimicrobial activity may be associated with increased interaction with microbial membranes, improved cellular uptake, Cu(II)/Cu(I)-mediated redox processes, oxidative stress and interactions with essential cellular proteins and nucleic acids. The observed antioxidant activity additionally demonstrates the redox-responsive nature of the complex, although direct mechanistic studies are required to establish the precise contribution of reactive oxygen species to antimicrobial action.

From a veterinary perspective, the findings identify the Cu(II)–quinoxaline complex as a promising in vitro lead compound for further investigation against animal-associated bacterial pathogens. Nevertheless, the present results should not be interpreted as evidence of clinical efficacy or immediate therapeutic applicability. Further studies involving mammalian-cell cytotoxicity, pharmacokinetics, acute and chronic toxicity, tissue distribution, copper accumulation, residue analysis, withdrawal-period determination and controlled in vivo efficacy are essential. Evaluation of antimicrobial-resistance development and environmental safety will also be important before practical veterinary applications are considered. Overall, the study demonstrates that quinoxaline-based copper coordination chemistry provides a useful platform for the development and investigation of alternative antimicrobial candidates within a veterinary.

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