I Research: Journal of Pharmaceutical Chemistry, 1 (1) (2026) 11-17
(0nline)

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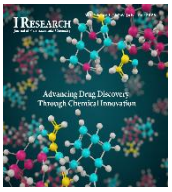

Synthesis, Spectral Characterization and *In-vitro* Biological Evaluation of a Novel Zn(II) Mixed-Ligand Complex of 5-Nitro-8-Hydroxyquinoline and L-Threonine

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ARTICLE INFO

Keywords:
Zn(II) complex
5-Nitro-8-hydroxyquinoline
L-Threonine
FTIR
Antioxidant activity
Antimicrobial activity

Article History:
Received : 27th September 2026
Accepted : 29th September 2026
Available online : 29th September 2026

ABSTRACT

Metal-based drug design continues to occupy a central position in medicinal chemistry because coordination of a bioactive ligand to a transition metal can markedly alter its pharmacological profile. In the present work, a novel Zn(II) mixed-ligand complex was synthesized from 5-nitro-8-hydroxyquinoline (a clinically used quinolone antimicrobial, also known as a nitroxoline analogue) and the essential amino acid L-threonine. 5-Nitro-8-hydroxyquinoline was first prepared from 8-hydroxyquinoline through nitrosation followed by oxidative nitration, purified with activated carbon, and then reacted with zinc acetate dihydrate and L-threonine in an aqueous-ethanolic medium to yield the target complex as a yellow precipitate. The ligand and the complex were characterized by FTIR and ^{1}H NMR spectroscopy, and the metal content was verified by atomic absorption spectroscopy (AAS). *In-vitro* antioxidant activity was assessed by the ferric reducing antioxidant power (FRAP) assay, while antifungal activity against Candida albicans and antibacterial activity against Escherichia coli and Staphylococcus aureus were evaluated by the well/cup diffusion method. FTIR spectra confirmed retention of the hydroxyl and aromatic ring systems together with a shift in the nitro-group stretching frequencies on complexation, while ^{1}H NMR spectra showed the expected aromatic proton pattern of the quinoline ring. The Zn(II) complex displayed concentration-dependent antioxidant activity and showed enhanced antifungal activity relative to the free ligand, producing a maximum zone of inhibition of 25 mm against C. albicans at 150 µg/mL, whereas the free ligand retained a marginally higher antibacterial effect against E. coli and S. aureus. These findings support the continued exploration of quinoline-amino acid Zn(II) complexes as multi-target antimicrobial and antioxidant leads.

Introduction

Medicinal chemistry sits at the interface of chemistry and pharmacy and is concerned with the design, synthesis and development of pharmaceutical agents. It draws on organic chemistry, biochemistry, physiology, microbiology, pharmacology, toxicology and computational modelling to explain how compounds are produced and how existing drugs behave in biological systems, including their structure-activity relationships [1]. While many early therapeutic agents were isolated directly from plants, the majority of medicines developed over the last several decades are of synthetic or semi-synthetic origin. Large-scale, systematic screening of newly synthesized organic molecules — although often inefficient on a per-compound basis — has nevertheless produced numerous lead compounds, and progress in spectroscopic and chromatographic techniques (NMR,mass spectrometry, X-ray crystallography, HPLC and related methods) has accelerated the discovery of new therapeutic entities.

Organic synthesis, the deliberate construction of target molecules from simpler precursors, underpins this process and extends into agrochemicals, polymers, materials science and cosmetics. Contemporary synthetic strategy increasingly incorporates green chemistry, catalysis and computational design to improve efficiency and sustainability, and methodological advances such as asymmetric synthesis, organometallic chemistry and photoredox catalysis continue to widen the range of accessible chemical space [2].

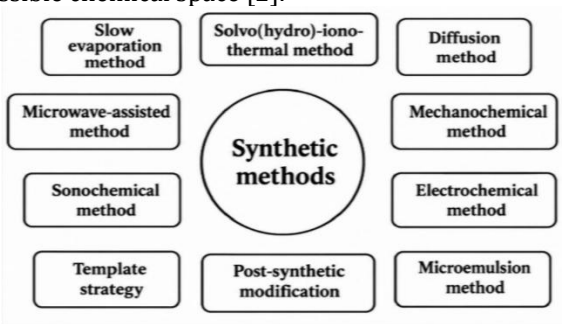

Figure 1. Overview of common synthetic methods used in the preparation of organic and coordination compounds.

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Metal-containing medicines occupy a distinctive niche within this landscape. Metal ions, together with the ligands and complexes they form, have shown notable inhibitory activity against cancer, diabetes, and bacterial and fungal infections, and their formation can be confirmed spectroscopically — for example, FTIR analysis is routinely used to establish complex formation [3]. Structure-activity relationships of such ligands and complexes can be tuned to maximize activity, since ligands and metal complexes frequently interact directly with cellular receptors [4]. Well-known clinical examples include platinum complexes that cross-link tumour DNA, gold complexes used in rheumatoid arthritis, lithium carbonate used as a mood stabilizer, zinc formulations that accelerate wound healing, silver-based dressings that limit burn infection, and lanthanum carbonate used in chronic kidney disease [5].

Ligands themselves are neutral or anionic species that donate an electron pair to a vacant metal orbital; depending on the number of donor atoms they may be mono-, bi- or tridentate, and the resulting complexes can adopt planar, tetrahedral, square-planar or octahedral geometries with diamagnetic or paramagnetic character. The geometry and electronic configuration of a complex directly influence its interaction with biological receptors and, therefore, its mechanism of action [6]. Amino-acid-based mixed-ligand complexes are of particular interest in this context because the amino acid can act as a secondary, biocompatible ligand that modulates the physicochemical and biological behaviour of the primary chelator [7,8].

8-Hydroxyquinoline and Nitroxoline

Quinoline and its 8-hydroxy derivative form a versatile pharmacophore in industrial and medicinal chemistry, having undergone extensive structural modification since the compound was first reported, and its derivatives display antibacterial, antioxidant and antidiabetic activity [9]. One clinically important derivative, 5-nitro-8-hydroxyquinoline (nitroxoline), is an antimicrobial agent first developed in the 1960s that has recently been revisited for drug-repurposing. It remains a first-line option for uncomplicated urinary tract infections caused by Enterobacterales such as Escherichia coli [10]. After oral administration it is rapidly absorbed, conjugated hepatically, and concentrated largely unchanged in the urine, which favours its urinary antimicrobial action; it has additionally shown *in-vitro* activity against drug-resistant mycobacteria (including Mycobacterium tuberculosis), Neisseria gonorrhoeae, Candida species (including C. auris), and the free-living amoeba Balamuthia mandrillaris [10].

L-Threonine

Amino acids are indispensable for growth and metabolism, contributing to gluconeogenesis, lipid synthesis and, at adequate dietary levels, to lifespan extension through mitochondrial and antioxidant pathways in animal models [11]. L-Threonine is an essential amino acid widely used in dietary and nutritional formulations; supplementation studies have shown it to be well tolerated, without adverse effects on anthropometric or biochemical parameters, while raising plasma concentrations of threonine and its metabolite L-2-aminobutyrate [12].

The aim of the work is to synthesize, characterize and biologically evaluate a novel Zn(II) complex of 5-nitro-8-hydroxyquinoline and L-threonine, with emphasis on its antioxidant, antibacterial and antifungal activity.

The objective includes Design, synthesize and optimize a Zn(II) mixed-ligand complex of 5-nitro-8-hydroxyquinoline and L-threonine, and determine its percentage yield. Characterize the complex by FTIR, ^{1}H NMR and atomic absorption spectroscopy (AAS) to confirm coordination and metal content.

Evaluate its *in-vitro* antioxidant (FRAP), antifungal and antibacterial activity against representative strains.

Materials and Methods

Drug Profile

The physicochemical profiles of the two key building blocks — the nitroso intermediate and the final quinoline ligand — together with the amino acid co-ligand, are summarized in Table 1 and Figures 2-4.

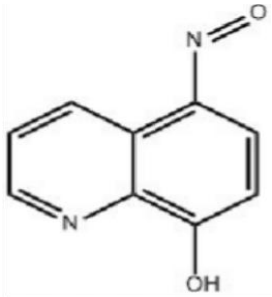

Figure 2. Chemical structure of 5-Nitroso-8-hydroxyquinoline (synthetic intermediate).

Property	5-Nitroso-8-hydroxyquinoline
IUPAC name	5-Nitrosoquinolin-8-ol
Molecular formula	C9H6N2O2
Molecular weight	174.16 g/mol
Melting point	245 ℃
Colour	Brownish green
Solubility	Soluble in DMSO/DMF; insoluble in water and ethanol

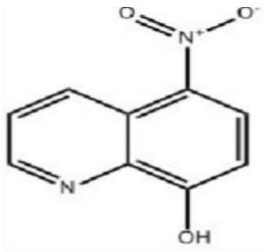

Figure 3. Chemical structure of 5-Nitro-8-hydroxyquinoline (nitroxoline; primary ligand).

Property	5-Nitro-8-hydroxyquinoline
IUPAC name	5-Nitroquinolin-8-ol
Molecular formula	C9H6N2O3
Molecular weight	190.16 g/mol
Melting point	179-183 ℃
Colour	Light yellow
Solubility	Soluble in DMSO, DMF and ethanol
Reported use	Urinary tract infection, biofilm disruption, UTI prophylaxis [10]

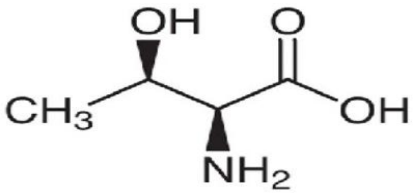

Figure 4. Chemical structure of L-Threonine (secondary/co-ligand).

Property	L-Threonine
IUPAC name	(2S,3R)-2-Amino-3-hydroxybutanoic acid
Molecular formula	C4H9NO3
Molecular weight	119.12 g/mol
Melting point	256 ℃
Colour	White crystalline powder
Solubility	Freely soluble in water; sparingly soluble in alcohol/ether

Synthesis

Preparation of 5-Nitroso-8-hydroxyquinoline: 8-Hydroxyquinoline (24.48 g, 0.5 mol) was suspended in stirred distilled water (222.4 mL) and treated with concentrated sulfuric acid (10 mL) at 15-18 °C. Sodium nitrite (12.23 g) in water (22.6 mL) was added dropwise over 30-40 min at 18-20 °C and the mixture held at this temperature for 3 h. After cooling, the reaction mixture was neutralized with 42% NaOH to pH 7.5-8.0 and then acidified with glacial acetic acid to pH 3.0-4.0. The resulting precipitate was filtered, washed with distilled water, and dried (percentage yield calculated as practical/theoretical × 100).

Figure 5. 5-Nitroso-8-hydroxyquinoline obtained after Step 1 of the synthesis.

Preparation of 5-Nitro-8-hydroxyquinoline: The nitroso-intermediate paste (15.2 g) in distilled water (63.3 mL) was oxidized with concentrated nitric acid (51.45 mL) at 35-40 °C for 2 h. The mixture was cooled to 5-10 °C, neutralized with 42% NaOH (pH 7.5-8.0), acidified with glacial acetic acid (pH 3.0-4.0), and the precipitate was washed free of nitrate/sulfate/acetate ions with distilled water followed by acetone, giving 5-nitro-8-hydroxyquinoline in 83.55% yield. The crude product was purified by reflux with activated carbon in acetone, filtration, and reprecipitation with water (75.28% recovery) [13].

Figure 6. 5-Nitro-8-hydroxyquinoline obtained after purification (Step 3).

Synthesis of the Zn(II) mixed-ligand complex: Zinc acetate dihydrate (219 mg) in distilled water (10 mL) was combined with 5-nitro-8-hydroxyquinoline (145 mg) in ethanol (10 mL), mixed and heated in a water bath for 10 min. An aqueous solution of L-threonine (1 mmol in 10 mL) was added slowly with constant stirring, heating continued to 50 °C, and dilute ammonia was added dropwise until a yellow precipitate of the Zn(II) complex formed. The mixture was cooled, filtered, washed successively with distilled water and ethanol, and dried under vacuum [14].

Figure 7. Zinc(II) mixed-ligand complex of 5-nitro-8-hydroxyquinoline and L-threonine (final product).

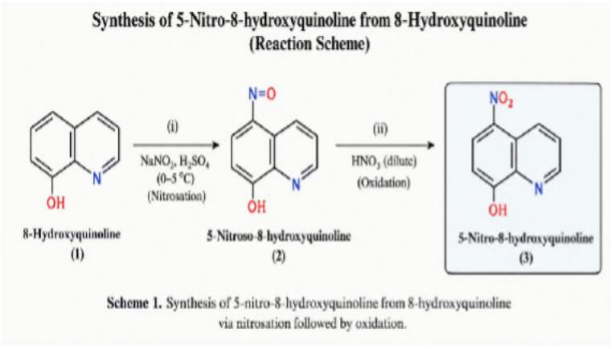

Figure 8. Proposed reaction scheme for formation of the Zn(II) complex.

Identification Tests

Preliminary identification of the intermediates and ligand was performed using standard colour-reaction tests: the resorcinol test and Liebermann's nitroso test for 5-nitroso-8-hydroxyquinoline, and the ferric chloride and copper sulfate tests for 5-nitro-8-hydroxyquinoline, each producing characteristic colour changes indicative of the respective functional groups [15-18].

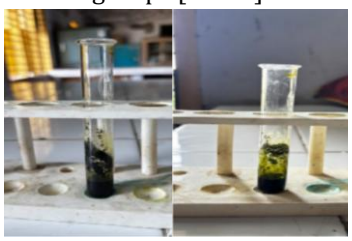

Figure 9. Colour reactions: (left) Resorcinol test; (right) Liebermann's nitroso test.

Figure 10. Colour reactions: (left) Ferric chloride test; (right) Copper sulfate test.

Spectroscopic Characterization

FTIR spectra of both synthetic intermediates and the final complex were recorded to track functional-group retention and coordination-induced shifts. ^{1}H NMR spectra were recorded to confirm the aromatic proton environment of the quinoline ring in the ligand and complex, and atomic absorption spectroscopy (AAS) was used to confirm the zinc content of the isolated complex.

In-vitro Antioxidant Activity (FRAP Assay)

Antioxidant capacity was measured by the ferric reducing antioxidant power (FRAP) assay, based on the ability of an antioxidant to reduce the Fe3+-ferricyanide complex to the intensely coloured Fe2+ form, read at 590 nm. Test solutions of the Zn(II) complex (50-250 µg/mL range in five levels) were combined with 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide, incubated at 50 °C for 20 min, treated with 10% trichloroacetic acid, centrifuged, and the supernatant reacted with 0.1% ferric chloride before absorbance measurement against ascorbic acid as the reference standard.

In-vitro Antifungal and Antibacterial Activity

Antifungal activity was assessed against Candida albicans by the agar well-diffusion method at graded concentrations (50, 100 and 150 µg/mL) against an untreated control, measuring the zone of inhibition (ZOI) in millimetres. Antibacterial activity of the ligand and the Zn(II) complex was evaluated against Escherichia coli and Staphylococcus aureus using the cup-plate diffusion method across three serial dilutions, with the mean ZOI recorded for each organism.

Results (Percentage Yield)

Step	Product	Percentage yield
1	5-Nitroso-8-hydroxyquinoline	84.97%
2	5-Nitro-8-hydroxyquinoline	83.55%
3	Purified 5-Nitro-8-hydroxyquinoline	75.28%

FTIR Spectral Analysis

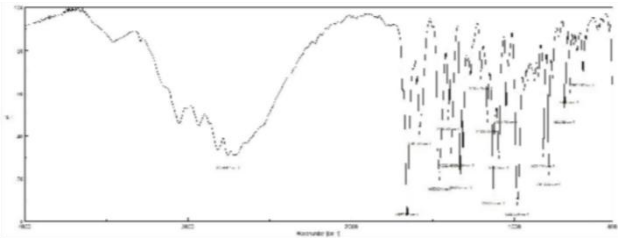

Figure 11. FTIR spectrum of 5-Nitroso-8-hydroxyquinoline.

Peak (cm-1)	Assignment	Interpretation
3360.7	O-H stretching	Hydroxyl group retained
2923.6	C-H stretching	Aromatic C-H vibration
1492.3	N=O stretching	Confirms nitroso group
1607.1	C=C stretching	Aromatic ring confirmed
1245.9	C-O stretching	Phenolic C-O bond retained

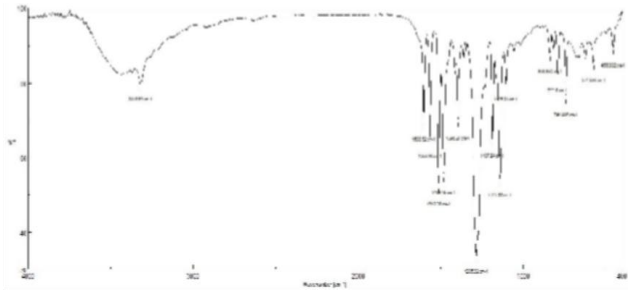

Figure 12. FTIR spectrum of 5-Nitro-8-hydroxyquinoline.

Peak (cm-1)	Assignment	Interpretation
3358.9	O-H stretching	Hydroxyl group retained
2922.5	C-H stretching	Aromatic C-H present
1605.4	C=C stretching	Aromatic ring retained
1534.2	N-O asym. stretching	Confirms nitro group
1346.8	N-O sym. stretching	Further confirms nitro substitution

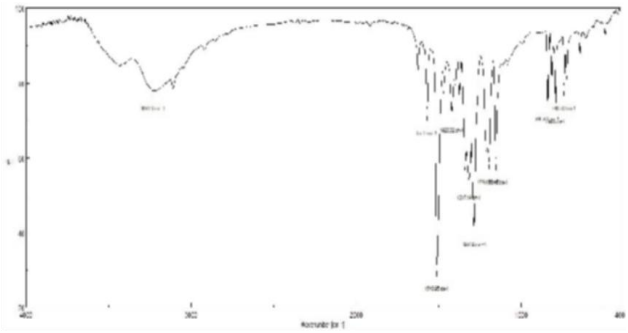

Figure 13. FTIR spectrum of the Zn(II) complex of 5-nitro-8-hydroxyquinoline and L-threonine.

Peak (cm-1)	Assignment	Interpretation
3362.4	O-H stretching	Hydroxyl group present
2924.8	C-H stretching	Aliphatic C-H bonds
1608.7	C=C stretching	Aromatic ring confirmed
1452.6	C-H bending	Aromatic/aliphatic bending
1248.3	C-O stretching	Phenolic C-O bond confirmed

^{1}H NMR Spectral Analysis

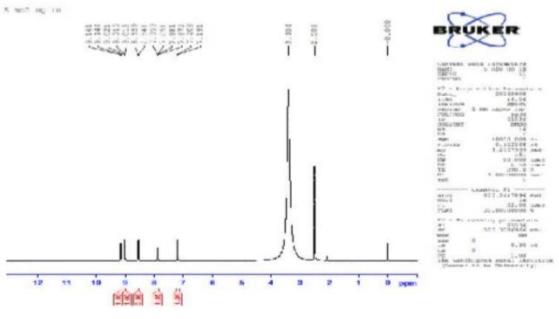

Figure 14. ^{1}H NMR spectrum of 5-Nitroso-8-hydroxyquinoline.

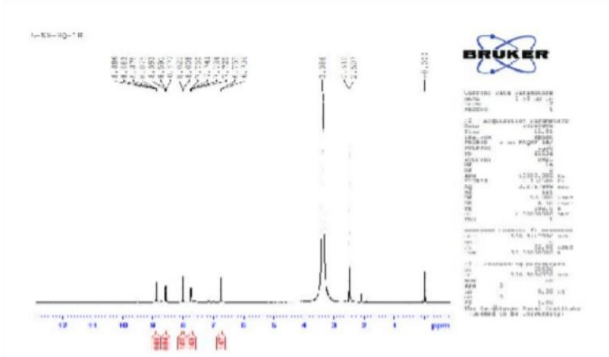

Figure 15. ^{1}H NMR spectrum of 5-Nitro-8-hydroxyquinoline.

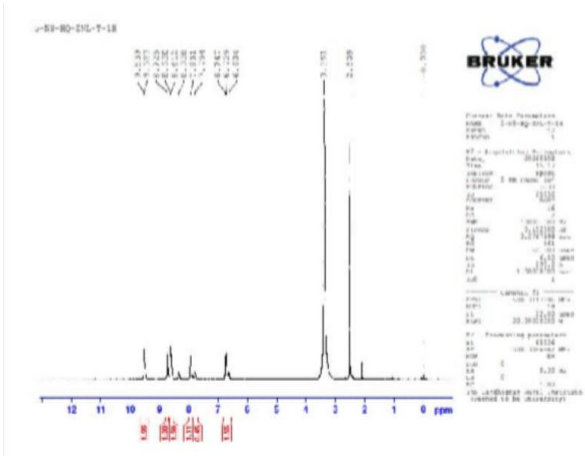

Figure 16. ^{1}H NMR spectrum of the Zn(II) complex.

Across the three spectra, five well-resolved downfield signals (δ 6.6-9.5 ppm) were consistently observed, matching the number of aromatic protons expected for the quinoline ring system. The overall multiplicity and integration pattern were retained after nitration and after complexation, while small shifts in chemical shift nd relative integration in the complex spectrum are consistent with a modified electronic environment upon Zn(II) coordination.

Atomic Absorption Spectroscopy (AAS)

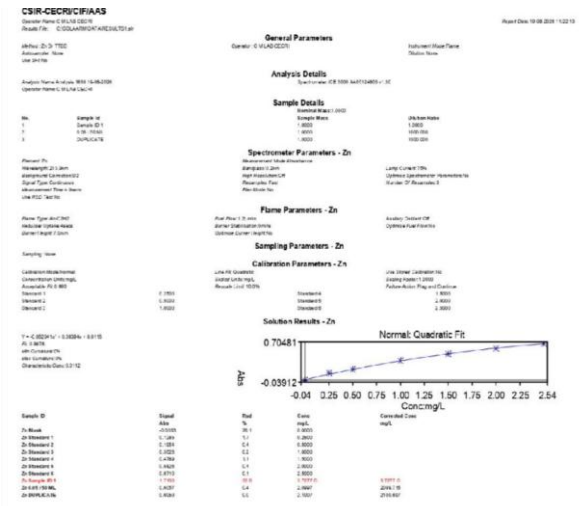

Figure 17. AAS trace confirming the zinc content of the synthesized complex.

In-vitro Antioxidant Activity

Absorbance of the test sample increased progressively from 0.25 to 0.49 across the five concentration levels, compared with 0.28-0.60 for the ascorbic acid standard (blank absorbance 0.11), confirming concentration-dependent reducing power (Figure 18).

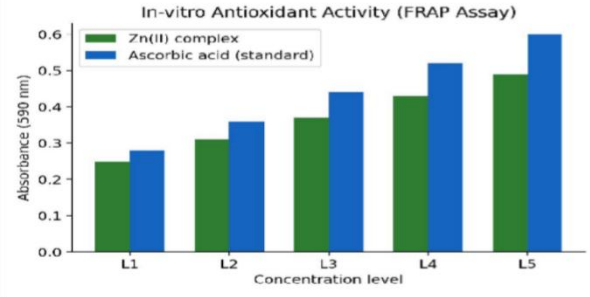

Figure 18. FRAP absorbance of the Zn(II) complex relative to the ascorbic acid standard across five concentration levels.

In-vitro Antifungal Activity

Both the free ligand and the Zn(II) complex produced concentration-dependent zones of inhibition against C. albicans, increasing from a 15 mm control to a maximum of 21 mm (ligand) and 25 mm (complex) at 150 µg/mL (Table 5, Figure 19).

Table 5: ***In-vitro Antifungal Activity***

Sample	Control	50 µg/mL	100 µg/mL	150 µg/mL
5-Nitro-8-hydroxyquinoline	15	18	20	21
Zn(II) complex	15	22	24	25

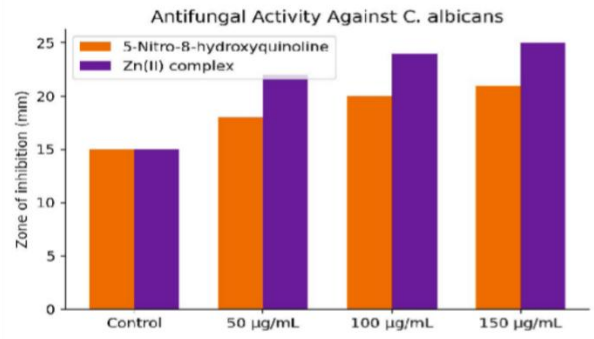

Figure 19. Zone of inhibition (mm) against C. albicans at increasing concentration.

In-vitro Antibacterial Activity

Organism	Sample	Dil. 1 (mm)	Dil. 2 (mm)	Dil. 3 (mm)	Mean ZOI (mm)
E. coli	5-Nitro-8-hydroxyquinoline	28.4	25.7	22.3	25.47
E. coli	Zn(II) complex	25.8	23.6	20.5	23.30
S. aureus	5-Nitro-8-hydroxyquinoline	26.2	24.5	21.8	24.17
S. aureus	Zn(II) complex	23.7	22.1	19.6	21.80

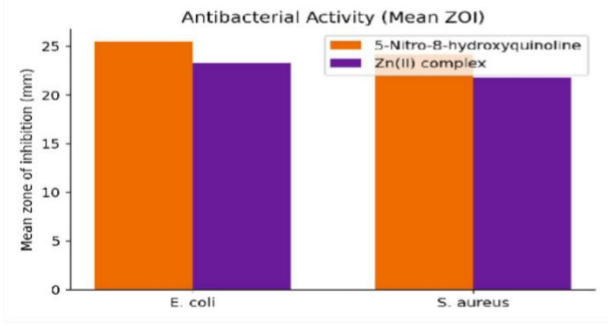

Figure 20. Mean zone of inhibition (mm) of the free ligand and Zn(II) complex against E. coli and S. aureus.

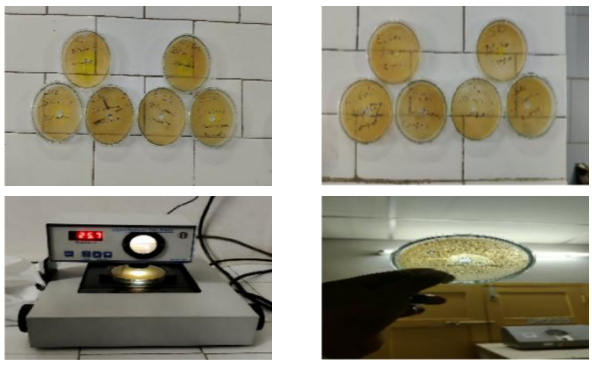

Figure 21. Representative zones of inhibition produced by the free ligand and the Zn(II) complex against the tested bacterial strains.

Result and Discussion

The FTIR data trace a coherent synthetic narrative: the hydroxyl and aromatic ring signals of 8-hydroxyquinoline persist throughout, the appearance of a distinct N=O stretch after nitrosation and its replacement by asymmetric/symmetric N-O stretches after nitration confirm successful conversion to the nitro derivative, and retention of the phenolic C-O stretch in the final complex — together with a shifted C-H bending mode — is consistent with zinc coordination through the available donor atoms rather than degradation of the ligand framework. The ^{1}H NMR spectra reinforce this picture: the aromatic proton count and general pattern of the quinoline ring are preserved from the nitroso-intermediate through to the complex, with only minor shift and integration changes on complexation, as expected for a ring system that remains intact while its immediate electronic environment is perturbed by metal binding.

Biologically, the Zn(II) complex showed a clear antioxidant response that increased with concentration, in line with previous reports that Zn(II) complexation with phenolic or chelating ligands can enhance radical-scavenging behaviour [19,20]. The complex also outperformed the free ligand in the antifungal assay, achieving a larger zone of inhibition against C. albicans at every tested concentration — a pattern consistent with reports that Zn(II) complexation improves antifungal

potency relative to free 8-hydroxyquinoline-type ligands [23]. By contrast, the free ligand retained a modest advantage over the complex in the antibacterial assay against both E. coli and S. aureus, mirroring the more variable, ligand- and strain-dependent trend for antibacterial activity described elsewhere in the amino-acid/quinoline-Zn(II) literature [8,9]. Together, these results suggest that Zn(II) complexation with 5-nitro-8-hydroxyquinoline and L-threonine selectively enhances antioxidant and antifungal behaviour while leaving antibacterial potency governed largely by the intact quinoline pharmacophore.

Conclusion

A novel Zn(II) mixed-ligand complex of 5-nitro-8-hydroxyquinoline and L-threonine was successfully synthesized in three well-defined steps with reproducible yields, and its formation was supported by FTIR, ^{1}H NMR and AAS data. The complex demonstrated concentration-dependent antioxidant activity and superior antifungal activity against C. albicans relative to the free ligand, while the free ligand retained comparatively stronger antibacterial activity against E. coli and S. aureus. These findings position the Zn(II)-nitroxoline-threonine complex as a promising multi-target candidate warranting further structural elucidation (e.g., single-crystal X-ray diffraction, elemental analysis, molar conductance and magnetic susceptibility studies) and expanded biological screening, including IC50 determination and evaluation against additional pathogenic strains.

Acknowledgement

Authors are thankful to K. M. College of Pharmacy, Madurai, Tamilnadu, India.

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