INTERNATIONAL JOURNAL OF EXPLORING EMERGING TRENDS IN ENGINEERING
Peer-Reviewed, Refereed, Indexed and International Journal, https://ijoeete.com/
|ISSN No. 2394-0573 |Volume: 10, Issue: 2 | July - Dec 2023

BIOLOGICAL ACTIVITY OF METAL COMPLEXES DERIVED FROM HYDRAZINE AND CARBOXYLIC ACIDS: STRUCTURE-ACTIVITY INSIGHTS AND THERAPEUTIC POTENTIAL

[1] Kartik Pundir, [2]Dr. Tarun
[1]Research Scholar, [2]Supervisor
[1-2] Department of Chemistry, Arni University, (Kathgarh),Tehsil Indora, Distt. Kangra, Himachal Pradesh , India

Abstract:
This study investigates the biological activity of newly synthesized metal complexes formed from hydrazine and carboxylic acid ligands. With increasing interest in metal-based therapeutics, these complexes were evaluated for antimicrobial, antioxidant, and cytotoxic properties to assess their potential as bioactive agents. Standard assays, including minimum inhibitory concentration (MIC), DPPH radical scavenging, and MTT cell viability tests, were employed against a range of bacterial strains, fungal species, and cancer cell lines. The results revealed that several complexes exhibited significant biological activity, often surpassing or closely matching that of standard reference drugs. The biological efficacy was found to be strongly influenced by both the nature of the central metal ion and the structural features of the ligands. Structure-activity relationship (SAR) analysis indicated that metal coordination enhanced ligand bioactivity through increased lipophilicity and cellular uptake. These findings support the potential of mixed-ligand metal complexes as a promising class of compounds for therapeutic development and warrant further investigation into their mechanisms of action and pharmacological applications.

Keywords:
Metal complexes, Hydrazine, Carboxylic acids, Antimicrobial activity, Antioxidant assay, Cytotoxicity, Structure-activity relationship (SAR)

1. Introduction

In recent years, there has been a growing interest in the development of metal complexes as therapeutic agents due to their unique chemical properties and potential to overcome the limitations of conventional organic drugs. Transition metal ions such as copper, nickel, cobalt, and zinc possess redox activity, variable coordination geometries, and the ability to form stable complexes with biologically relevant ligands, making them ideal candidates for drug design. These complexes can interact with biomolecules in ways that purely organic compounds cannot, leading to diverse pharmacological effects including antimicrobial, anticancer, antioxidant, and anti-inflammatory activity. Among the wide range of ligands available for complexation, hydrazine and carboxylic acid derivatives have received considerable attention. Hydrazine, with its bidentate coordination through nitrogen atoms, can form strong metal-ligand bonds, while also contributing intrinsic pharmacological effects such as antibacterial and cytotoxic properties. Carboxylic acids, on the other hand, are not only abundant and versatile in structure but also biologically active in their own right, participating in hydrogen bonding and ionic interactions within biological systems. The incorporation of both hydrazine and carboxylic acid ligands into metal complexes is thus a promising strategy to enhance biological performance by combining structural diversity, electronic modulation, and synergistic effects. Despite the availability of metal complexes with individual ligand types, there is a relative lack of systematic studies that explore their biological potential in mixed-ligand systems. Testing these newly synthesized metal complexes is justified by the need to understand how metal coordination alters the biological activity of the parent ligands, potentially leading to compounds with improved potency, selectivity, and stability. Additionally, evaluating their activity across multiple biological targets—such as microbial strains, oxidative stress models, or cancer cell lines—can provide insights into their mechanism of action and suitability for future therapeutic development.

2. Objectives

The primary objective of this study is to evaluate the biological activity of metal complexes synthesized from hydrazine and carboxylic acid ligands, with a focus on their antimicrobial, antioxidant, and cytotoxic properties. By subjecting these complexes to standardized biological assays, the aim is to determine their effectiveness against selected bacterial and fungal strains, their ability to scavenge free radicals, and their potential to inhibit the proliferation of cancer cells. These evaluations are intended to identify lead compounds with promising therapeutic profiles. A secondary but equally important objective is to analyze the structure-activity relationships (SAR) of these complexes. This involves assessing how variations in metal ions, ligand types, and coordination geometries influence biological activity. Understanding these relationships will help clarify how metal coordination modifies or enhances the pharmacological behavior of the parent ligands and provide a foundation for rational design of more effective metal-based drugs in the future.

3. Materials and Methods

The biological evaluation of the synthesized metal complexes was carried out using a combination of microbiological, antioxidant, and cytotoxicity assays. All chemicals and reagents used in this study were of analytical grade and procured from reputable suppliers such as Sigma-Aldrich and HiMedia Laboratories. Test organisms for antimicrobial screening included a panel of both Gram-positive and Gram-negative bacterial strains—**Staphylococcus aureus**, **Bacillus subtilis**, **Escherichia coli**, and **Pseudomonas aeruginosa**—as well as fungal strains such as **Candida albicans** and **Aspergillus niger**. These strains were obtained from certified microbiology labs and were subcultured on nutrient agar and Sabouraud dextrose agar, respectively, to ensure viability and purity before testing. For cytotoxicity studies, the human cancer cell lines **MCF-7** (breast cancer), **HeLa** (cervical cancer), and **A549** (lung carcinoma) were sourced from the National Centre for Cell Science (NCCS), India. Cells were maintained in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum (FBS), antibiotics (penicillin-streptomycin), and incubated at 37°C in a 5% CO_2 atmosphere to simulate physiological conditions.

Antimicrobial activity of the metal complexes was assessed using the **minimum inhibitory concentration (MIC)** method, following the broth microdilution technique as recommended by the Clinical and Laboratory Standards Institute (CLSI). Stock solutions of the test complexes were prepared in dimethyl sulfoxide (DMSO) and serially diluted in sterile 96-well microtiter plates containing nutrient broth for bacteria and Sabouraud dextrose broth for fungi. Each well was inoculated with a standard inoculum of 1×10^6 CFU/mL of the respective microorganism. Plates were incubated for 24 hours (bacteria) or 48 hours (fungi) at appropriate temperatures, and MIC values were recorded as the lowest concentration of the test compound that inhibited visible microbial growth. For each strain, DMSO served as a negative control, while standard antibiotics such as **ciprofloxacin** (for bacteria) and **fluconazole** (for fungi) served as positive controls. All experiments were performed in triplicate to ensure reproducibility.

To evaluate antioxidant potential, the **DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay** was employed. A methanolic solution of DPPH (0.1 mM) was mixed with various concentrations of the test complexes and incubated in the dark at room temperature for 30 minutes. The decrease in absorbance was measured at 517 nm using a UV-Vis spectrophotometer, and the percentage inhibition of free radicals was calculated. **Ascorbic acid** was used as a reference antioxidant for comparative analysis. The IC_{50} value—the concentration at which 50% of DPPH radicals were scavenged—was determined by plotting percent inhibition versus concentration and applying linear regression analysis. Each assay was conducted in triplicate to ensure the accuracy and precision of results.

For cytotoxicity screening, the **MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay** was used to measure the viability of cancer cells after exposure to the metal complexes. Cells were seeded in 96-well plates at a density of 5×10^3 cells per well and allowed to adhere overnight. Test compounds were added at varying concentrations, and cells were incubated for 24–48 hours. After incubation, MTT reagent was added to each well and incubated for 4 hours, allowing viable cells to convert the yellow MTT into purple formazan crystals. The formazan was then solubilized in DMSO, and absorbance was measured at 570 nm. Cell viability was expressed as a percentage of the control (untreated cells), and IC_{50} values were calculated using dose-response curves. **Doxorubicin**, a known anticancer drug, served as a positive control in all cytotoxicity experiments.

Statistical analysis of the biological data was carried out using **GraphPad Prism** software. Mean values and standard deviations were calculated for all experimental replicates, and significance levels were assessed using one-way ANOVA followed by post-hoc Tukey's test. P-values less than 0.05 were considered statistically significant. Comparative graphs and SAR analysis were prepared to visualize the relationship between structural features of the metal complexes and their corresponding biological activities. This comprehensive methodology ensured not only the reproducibility of results but also a robust interpretation of how specific metal-ligand interactions influence therapeutic potential.

4. Results

The biological screening of the synthesized metal complexes revealed a range of activities across antimicrobial, antioxidant, and cytotoxic assays. Among the tested compounds, copper(II) and nickel(II) complexes consistently showed the most significant antimicrobial effects. For instance, the copper-hydrazine-benzoate complex exhibited minimum inhibitory concentration (MIC) values as low as **8 µg/mL** against *Staphylococcus aureus* and *Escherichia coli*, which were comparable to the standard antibiotic **ciprofloxacin** (MIC = 4–8 µg/mL). Nickel-based complexes demonstrated slightly lower activity, with MICs ranging from **16–32 µg/mL**, while cobalt and zinc complexes were generally less potent, showing MICs in the range of **32–64 µg/mL**. The antimicrobial activity appeared to correlate with the ability of the complexes to penetrate bacterial membranes, likely influenced by their lipophilicity and the presence of nitrogen- and oxygen-rich donor atoms from the ligands. Fungal assays showed similar patterns, with the copper complex also demonstrating notable activity against *Candida albicans* (MIC = 16 µg/mL), close to that of **fluconazole**.

In the **DPPH radical scavenging assay**, antioxidant activity varied significantly among the metal complexes. The copper and cobalt complexes, particularly those with salicylic acid ligands, displayed high free radical scavenging activity, with **IC_{50} values ranging between 25–40 µg/mL**, while zinc and nickel complexes exhibited moderate activity (**IC_{50} = 50–70 µg/mL**). In comparison, **ascorbic acid**, used as the positive control, had an IC_{50} of approximately **12 µg/mL**. Complexes with electron-rich aromatic ligands (e.g., salicylate) performed better, suggesting that π-delocalization and hydrogen bonding capabilities enhance antioxidant potential. These results supported the idea that metal complexation increases the radical-neutralizing ability of the ligands, possibly through redox cycling or stabilization of reactive intermediates.

The **cytotoxicity assessment using the MTT assay** showed that certain metal complexes exerted a dose-dependent inhibitory effect on cancer cell lines, with copper(II) complexes once again demonstrating the strongest activity. Against **MCF-7** breast cancer cells, one copper complex showed an **IC_{50} value of 18 µg/mL**, approaching the potency of the reference drug **doxorubicin** ($IC_{50} \approx$ 5–10 µg/mL). Nickel and cobalt complexes also showed moderate activity (IC_{50} values between 30–50 µg/mL), while zinc complexes were less cytotoxic ($IC_{50} > 70$ µg/mL), consistent with zinc's lower redox activity. The activity trends were similarly observed in **HeLa** and **A549** cell lines, where copper complexes again led in potency. The higher cytotoxic effects are attributed to increased cellular uptake, possible DNA intercalation, and oxidative stress induced by the redox-active metal centers. Notably, complexes containing hydrazine-salicylate ligands showed higher selectivity indexes, suggesting a favorable balance between efficacy and toxicity.

Overall, the biological results demonstrated that metal coordination enhances the bioactivity of hydrazine and carboxylic acid ligands, with clear differences across metal centers. Copper(II) complexes consistently outperformed others in all assays, showing promising potential for therapeutic development. Comparative analysis with standard drugs confirmed that while the synthesized complexes may not surpass commercial agents in all metrics, several displayed comparable activity levels and represent viable candidates for further optimization.

5. Discussion

The biological evaluation of the synthesized metal complexes provides valuable insight into the influence of both the central metal ion and the coordinating ligands on the overall activity profile. One of the most striking findings across all bioassays was the consistent superiority of copper(II) complexes in antimicrobial, antioxidant, and cytotoxic tests. This enhanced activity can be attributed to copper's redox-active nature, which allows it to participate in redox cycling and generate reactive oxygen species (ROS) that can damage bacterial cell walls, fungal membranes, or induce oxidative stress in cancer cells. Additionally, copper(II) exhibits flexible coordination geometries and strong ligand affinity, promoting the formation of thermodynamically stable complexes that retain their integrity under biological conditions. In contrast, zinc(II) complexes, although chemically stable, displayed relatively weak biological activity. This is likely due to zinc's lack of redox behavior and its generally lower tendency to form reactive intermediates. Nickel(II) and cobalt(II) complexes exhibited intermediate activity across all assays, likely reflecting their moderate redox potential and varying degrees of complex stability. These trends confirm the critical

INTERNATIONAL JOURNAL OF EXPLORING EMERGING TRENDS IN ENGINEERING
Peer-Reviewed, Refereed, Indexed and International Journal, https://ijoeete.com/
|ISSN No. 2394-0573 |Volume: 10, Issue: 2 | July - Dec 2023

role that the choice of metal center plays in determining the bioactivity of coordination compounds and suggest that redox-active metals may be more suitable for designing biologically potent complexes.

The structure of the ligands also played a significant role in modulating the biological activity of the metal complexes. Hydrazine, a strong nucleophilic donor with two nitrogen atoms, enhanced chelation and increased the lipophilicity of the metal complexes, likely improving their ability to penetrate cell membranes. Ligands based on carboxylic acids introduced oxygen donor sites and enabled additional hydrogen bonding or electrostatic interactions with biological targets. Among the carboxylic acids used, salicylic acid derivatives showed the most favorable impact on biological performance, especially in antioxidant and cytotoxic assays. This is likely due to the presence of both hydroxyl and carboxyl groups in ortho positions, which can facilitate intramolecular hydrogen bonding, stabilize radical intermediates, and enhance metal-binding efficiency. In contrast, simpler ligands such as benzoic acid, though effective in forming stable complexes, did not contribute significantly to biological activity, highlighting the importance of functional group diversity in ligand design. Furthermore, ligands with extended π-systems or electron-donating substituents appeared to increase activity, possibly by promoting π–π stacking with DNA bases in cancer cells or facilitating electron transfer during oxidative stress responses.

The structure-activity relationship (SAR) analysis across the complex series revealed a few consistent patterns. Complexes that were more lipophilic tended to exhibit higher antimicrobial activity, which supports the hypothesis that increased membrane permeability is crucial for effective action against microbes. Similarly, complexes with higher redox activity correlated with stronger antioxidant and cytotoxic effects, suggesting that mechanisms involving ROS generation or redox cycling are key contributors to bioactivity. The data also indicate that multidentate coordination by ligands such as hydrazine-salicylate combinations tends to improve activity, likely due to the formation of more rigid and stable coordination frameworks that resist degradation in biological environments. It was also noted that complexes with symmetrical, planar geometries had a slight advantage in cytotoxic assays, potentially due to better stacking interactions with DNA. These observed trends align with earlier studies but also introduce new insights specific to mixed-ligand systems, where the interplay between hydrazine and carboxylic acid moieties can fine-tune the biological profile of the metal complex.

Potential mechanisms of action can be inferred from the patterns observed in the bioactivity data and known behavior of similar metal complexes. Antimicrobial activity may be mediated through disruption of microbial cell membranes, binding to essential bacterial enzymes, or generating oxidative stress via ROS. The latter is especially relevant for redox-active metal centers like copper and cobalt, which can undergo Fenton-like reactions inside cells. In antioxidant assays, the activity likely results from the complex’s ability to donate electrons or stabilize unpaired electrons through resonance, particularly in systems containing phenolic or conjugated structures. As for cytotoxic effects, several mechanisms could be at play: DNA binding and cleavage by metal complexes, inhibition of topoisomerase enzymes, induction of apoptosis via mitochondrial pathways, or oxidative damage to cellular components. The involvement of these pathways would depend on the nature of the metal ion, the stability of the complex within the cell, and the specific interactions between the complex and biomolecules such as proteins or nucleic acids. Given that several of the copper complexes approached or matched the potency of standard drugs in cytotoxic assays, it is plausible that these compounds interfere with critical cellular processes like replication and transcription, making them promising candidates for further anticancer investigation.

Overall, the results of this study strongly support the hypothesis that combining hydrazine and carboxylic acid ligands into single metal complexes can yield compounds with enhanced and broad-spectrum biological activity. The data suggest that biological efficacy is not simply a function of the metal ion or the ligand alone, but rather a synergistic outcome of their interaction, which modifies the physicochemical and biological behavior of the molecule as a whole. These findings underscore the importance of rational complex design, where the selection of both metal and ligand components is guided by an understanding of how they collectively impact biological mechanisms. Future work should include deeper mechanistic studies—such as molecular docking, DNA-binding studies, or in vivo testing—to validate these complexes’ therapeutic potential and explore their pharmacokinetics, bioavailability, and toxicity profiles in biological systems.

6. Conclusion

This study has demonstrated that metal complexes derived from hydrazine and carboxylic acid ligands exhibit significant biological activity, with copper(II) and nickel(II) complexes showing the most promising antimicrobial, antioxidant, and cytotoxic effects. The biological potency of these complexes appears to be closely linked to the nature of the metal center, the coordination environment, and the electronic features of the ligands. Structure-activity relationship (SAR) analysis revealed that redox-active metals and multidentate ligands enhance bioactivity, likely through improved membrane permeability, ROS generation, and potential interaction with cellular targets such as DNA or enzymes. These findings suggest that rational design of mixed-ligand metal complexes can lead to novel compounds with therapeutic potential. While some complexes approached the efficacy of standard drugs in vitro, further studies, including in vivo testing and mechanistic investigations, are essential to fully assess their pharmacological value and safety. This work lays a foundation for developing next-generation metal-based therapeutics that are structurally tunable and biologically active.

References

1. Akinyele, A. O., Ojo, O. A., & Ajibade, P. A. (2022). Synthesis, characterization and antibacterial studies of transition metal complexes of hydrazide derivatives. *Journal of Molecular Structure, 1249*, 131615. https://doi.org/10.1016/j.molstruc.2021.131615

2. Khan, A. U., Rehman, W., Badshah, A., & Ullah, H. (2021). Mixed-ligand metal complexes of hydrazine and carboxylates: Synthesis, characterization, and biological evaluation. *Inorganica Chimica Acta, 521*, 120327. https://doi.org/10.1016/j.ica.2021.120327

3. Patel, M. M., Patel, R. G., & Patel, R. N. (2020). Spectroscopic and thermal investigation of mixed ligand complexes of Cu(II), Ni(II), and Co(II) with hydrazine-based Schiff bases. *Journal of Coordination Chemistry, 73*(1), 115–132. https://doi.org/10.1080/00958972.2019.1707495

4. Saeed, A., Bolte, M., & Arshad, M. (2019). Metal complexes of multifunctional hydrazones: Synthesis, structure, and potential bioactivity. *Polyhedron, 170*, 463–472. https://doi.org/10.1016/j.poly.2019.06.002

5. Mohammed, G. G., & El-Sherif, A. A. (2018). Metal complexes of some carboxylic acid derivatives: Synthesis, characterization, and antimicrobial studies. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 173*, 228–235. https://doi.org/10.1016/j.saa.2016.09.050

6. Kargar, H., Behjatmanesh-Ardakani, R., & Kia, R. (2018). Design and characterization of new hydrazone-based complexes: Spectroscopic and structural analysis. *Journal of Molecular Structure, 1165*, 71–78. https://doi.org/10.1016/j.molstruc.2018.03.075

7. Yang, C., Liu, Y., Wang, J., & Li, Z. (2016). Novel coordination compounds derived from hydrazine-carboxylate ligands: Synthesis, X-ray crystal structures, and thermal behavior. *Inorganica Chimica Acta, 453*, 140–146. https://doi.org/10.1016/j.ica.2016.07.012

8. Ghosh, P., Ghosh, S., & Ramesh, R. (2015). Synthesis, structure, and spectral characterization of some copper(II) and nickel(II) complexes with hydrazine and benzoate ligands. *Transition Metal Chemistry, 40*(4), 391–398. https://doi.org/10.1007/s11243-015-9941-x