**Title:**

# Synthesis, Characterization, Biological Evaluation, and Theoretical Insights of a Novel Zinc(II) Carboxylate Complex

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## Abstract

A novel zinc(II) carboxylate complex [$Zn(L)_2$], where L represents the 9H-fluorene-9-carboxylate ligand, was synthesized and characterized through FTIR, $^{1}H$ and $^{13}C$ NMR spectroscopy, elemental analysis, and atomic absorption spectroscopy. The spectral data confirmed bidentate coordination via carboxylate oxygen atoms, and the complex exhibited high thermal stability with a melting point above 280 °C. Biological evaluation revealed moderate antioxidant activity in total reducing power (32.1 µg AAE/mg), total antioxidant capacity (22.2 µg AAE/mg), and DPPH radical scavenging assays (15.7% at 50 µg/mL). Cytotoxic activity was observed against *Artemia salina* with 35% lethality at 50 µg/mL, while hemolytic activity reached 64.25% at 100 µg/mL, indicating potential membrane-disruptive effects. Computational electrostatic potential (ESP) analysis showed negative charge localization over carboxylate groups and a positively charged zinc center, highlighting potential reactive sites. The complex also demonstrated promise for non-biological applications as a Lewis acid catalyst and thermal stabilizer. This study integrates synthetic, biological, and theoretical approaches to explore the multifunctional potential of zinc(II) coordination complexes.

**Keywords:** Zinc(II) complex, carboxylate ligand, antioxidant activity, cytotoxicity, hemolysis, electrostatic potential, coordination chemistry, catalysis, NMR spectroscopy

## Section 1: Introduction

Zinc, a biologically essential element, plays a pivotal role in numerous physiological processes due to its involvement in enzymatic catalysis, gene expression, and immune response regulation. Among the transition metals, zinc(II) ions are particularly intriguing for coordination chemistry due to their fully filled d-orbitals ($d^{10}$ configuration), which confer a lack of ligand field stabilization energy but allow for flexible coordination geometries and stable complex formation. Over the past few decades, zinc(II) complexes have garnered considerable attention for both their biological and industrial applications, including antimicrobial, antioxidant, anticancer, and catalytic uses (Niaz et al., 2019; Zubair et al., 2018).

In coordination chemistry, carboxylate ligands have long served as reliable and versatile chelating agents due to their ability to coordinate through oxygen atoms, thereby enhancing the structural and chemical stability of resulting metal complexes. Aromatic carboxylates, such as 9H-fluorene-9-carboxylic acid, are particularly attractive for complexation due to their rigid planar structures and extended conjugation, which often facilitate π–π interactions and potential DNA binding. When combined with Zn(II), these ligands contribute to the formation of robust complexes with interesting spectroscopic and functional properties (Kaleem et al., 2019).

The primary focus of the present study is the synthesis, structural characterization, and application profiling of a novel Zn(II) complex, referred to as **Complex 1**, with the general formula $[Zn(L)_2]$, where L is the 9H-fluorene-9-carboxylate anion. The synthesis was carried out using standard coordination procedures, and the complex was characterized by various analytical and spectroscopic techniques, including elemental analysis, Fourier-transform infrared spectroscopy (FTIR), and nuclear magnetic resonance ($^{1}H$ and $^{13}C$ NMR) spectroscopy. The data confirmed the successful formation of the complex and provided insights into the coordination environment around the Zn(II) center.

**Biological Significance of Zinc Complexes**

Zinc-based coordination compounds are extensively investigated for their therapeutic potential. Their relatively low toxicity compared to other transition metal complexes, combined with their ability to mimic the structural and catalytic features of metalloenzymes, makes them ideal candidates for biomedical applications. In particular, zinc complexes have been evaluated for their ability to function as antioxidant agents, DNA intercalators, and cytotoxic agents against tumor cell lines (Aslam et al., 2022; Zubair et al., 2018).

In the current research, Complex 1 was assessed for its antioxidant capacity using three standard methods: total reducing power (TRP), total antioxidant capacity (TAC), and DPPH radical scavenging assay. These tests revealed moderate antioxidant potential, suggesting that the complex can mitigate oxidative stress, a known contributor to various pathological conditions, including cancer and neurodegenerative disorders. Furthermore, its cytotoxic properties were assessed using the brine shrimp lethality assay, which is a well-established preliminary screening tool for detecting broad-spectrum bioactivity. Hemolytic activity tests further supported the compound's interaction with biological membranes, indicating potential for drug delivery or antimicrobial mechanisms.

**Non-Biological Relevance and Industrial Potential**

Beyond biological significance, zinc complexes also hold great promise in non-biological domains. Zinc carboxylates are widely used as catalysts in organic synthesis, notably in transesterification and ester hydrolysis reactions, due to the Lewis acidic nature of the Zn(II)

center. Moreover, their thermal stability renders them useful as stabilizers in polymer processing and as precursors in material science applications (Farooq et al., 2020). Although the primary aim of this study is to examine biological relevance, preliminary suggestions and rationalizations regarding the catalytic potential of Complex 1 are also included, paving the way for future investigations.

Zinc complexes derived from aromatic carboxylates also exhibit potential as intermediates in the formation of functional materials, such as metal-organic frameworks (MOFs) and supramolecular architectures. Their rigid ligands can impart directional bonding features conducive to framework assembly. These materials, in turn, have applications in gas storage, separation, and catalysis. Given the stability and structural features of Complex 1, it may be postulated that such a compound could serve as a structural unit in hybrid materials or layered assemblies.

**Integration of Computational Chemistry**

In contemporary coordination chemistry, computational modeling serves as a vital tool in elucidating the electronic structure, charge distribution, and reactivity patterns of metal complexes. For this study, electrostatic potential (ESP) mapping was performed using Gasteiger charge approximation to identify nucleophilic and electrophilic regions within Complex 1. While high-level Density Functional Theory (DFT) methods remain the gold standard for molecular orbital and geometry optimization analyses, the limited computational complexity of the current system allows for an approximate but insightful ESP analysis.

The partial charge distribution revealed that electron-rich sites are predominantly localized over the carboxylate oxygen atoms and aromatic systems, suggesting these regions as active sites for interactions with biomolecules such as DNA or proteins. Such mapping also assists in predicting hydrogen bonding patterns and possible docking behavior in ligand-receptor binding simulations. This computational perspective adds a theoretical validation to the experimentally observed biological behavior and further supports the suggested non-biological roles in catalysis and material formation.

**Previous Literature and Research Gap**

Numerous studies have addressed the biological potential of zinc(II) coordination complexes. For instance, Zubair et al. (2018) synthesized and evaluated a series of Zn(II) complexes with Schiff base ligands for their antibacterial and anticancer properties. Niaz et al. (2019) investigated zinc complexes with various carboxylate ligands and reported their radical scavenging and cytotoxic capabilities. Similarly, Kaleem et al. (2019) explored the structural, spectroscopic, and bioactive behavior of Zn(II) coordination compounds, noting their moderate efficacy and proposing further structural refinements for enhanced potency.

Despite this progress, a considerable research gap persists in integrating non-biological perspectives, such as thermal behavior, catalytic activity, and theoretical modeling, into the study of zinc complexes. Often, studies are limited to isolated bioassays without exploring the broader physicochemical or industrial implications. Moreover, very few reports contextualize their findings with computational descriptors that can complement empirical data. This study, therefore, addresses this gap by proposing a multi-dimensional assessment model that combines empirical synthesis and biological testing with computational validation and non-biological application potential.

**Objectives of the Study**

This research aims to synthesize and characterize a new Zn(II) carboxylate complex, $[Zn(L)_2]$, and to explore its multifaceted properties. The specific objectives include:

1. To synthesize and characterize the zinc(II) complex using FTIR, $^{1}H$, and $^{13}C$ NMR spectroscopy.
2. To evaluate the biological activity of the complex, including antioxidant, cytotoxic, and hemolytic assays.
3. To suggest potential non-biological applications such as catalysis and thermal stabilization based on the structural features.
4. To perform computational electrostatic potential mapping for understanding charge distribution and reactivity.
5. To integrate the findings into a comprehensive understanding of the complex's utility in both biomedical and industrial domains.

**Section 2: Experimental**

**Chemicals and Reagents**

All chemicals and solvents used in the synthesis and analysis of zinc(II) complexes were of analytical grade and used without further purification. Zinc acetate dihydrate, 9H-fluorene-9-carboxylic acid, sodium hydroxide, and solvents such as methanol, ethanol, acetone, chloroform, and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (USA) and Merck (Germany). Deuterated DMSO (DMSO-$d_6$) was used as an NMR solvent. Pyridine, 2,2'-bipyridine, and 1,10-phenanthroline were used as co-ligands for comparison in extended complex formation. All solutions were prepared using double-distilled water.

**Instrumentation**

**Melting Points** were determined in open capillaries using an Electrothermal digital melting point apparatus.

**FTIR Spectra** were recorded on a Bruker Alpha FTIR spectrophotometer using KBr pellets in the range 4000–400 $cm^{-1}$.

**$^{1}H$ and $^{13}C$ NMR Spectra** were acquired using a Bruker Avance 500 MHz NMR spectrometer in DMSO-$d_6$ with tetramethylsilane (TMS) as an internal standard.

**Elemental Analysis (CHN)** was conducted using a PerkinElmer 2400 Series II elemental analyzer.

**Atomic Absorption Spectroscopy (AAS)** was used to determine the zinc content using a PerkinElmer Analyst 400 spectrophotometer.

**Synthesis of Ligand and Complex**

**Preparation of Sodium 9H-fluorene-9-carboxylate (NaL)**

Sodium salt of the ligand was prepared by dissolving 5 mmol of 9H-fluorene-9-carboxylic acid in water and adding dropwise 5 mmol of sodium hydroxide under constant stirring. The reaction mixture was stirred at room temperature until a clear solution was formed. The resulting sodium salt was obtained as a white solid by rotary evaporation and dried under vacuum.

**Synthesis of $[Zn(L)_2]$ (Complex 1)**

To a methanolic solution (20 mL) of the sodium ligand (5 mmol), a warm methanolic solution (20 mL) of zinc acetate dihydrate (2.5 mmol) was added dropwise with continuous stirring. The reaction mixture was maintained at 50 °C for 2 hours. Upon formation of a precipitate, the solid was filtered, washed with cold methanol and distilled water, and air-dried.

**Yield:** 85%;
**Molecular formula:** $C_{28}H_{18}O_4Zn$;
**Melting point:** 283–285 °C;
**Elemental Analysis (Calc/Found):** C: 69.51/69.52%, H: 3.75/3.74%;
**Zn content (Calc/Found):** 13.51/11.93% (via AAS)

**Spectroscopic Characterization**

**FTIR Analysis**

FTIR spectrum of Complex 1 showed a significant shift in the stretching frequencies of the carboxylate group, confirming coordination. The broad band around 3400 $cm^{-1}$ was attributed to O–H stretching. Symmetric and asymmetric stretching bands of $COO^-$ appeared near 1580 and 1400 $cm^{-1}$, respectively, indicating bidentate coordination (Silverstein et al., 2014).

**$^{1}$H NMR Spectroscopy**

The $^{1}$H NMR spectrum displayed multiple peaks in the aromatic region (δ = 7.27–7.91 ppm) corresponding to the fluorene moieties. The downfield shift and splitting patterns were consistent with symmetrical coordination around Zn(II), indicating retention of aromatic structure with slight deshielding due to metal–ligand interaction.

**$^{13}$C NMR Spectroscopy**

The $^{13}$C NMR spectrum revealed peaks between δ = 120–144 ppm for aromatic carbons and a distinctive peak near 175.3 ppm corresponding to the coordinated carboxyl carbon. The upfield shifts compared to free ligand suggested electron withdrawal from the ligand due to coordination with Zn(II).

**Biological Assays**

**Antioxidant Activity**

**Total Reducing Power (TRP):**
This assay was based on the reduction of $Fe^{3+}$ to $Fe^{2+}$ in the presence of antioxidant compounds. The sample absorbance at 630 nm was used to calculate ascorbic acid equivalents (µg AAE/mg). Complex 1 demonstrated a TRP of 32.1 µg AAE/mg.

**Total Antioxidant Capacity (TAC):**
Based on the reduction of Mo(VI) to Mo(V), the green phosphate/Mo(V) complex was quantified spectrophotometrically at 630 nm. Complex 1 exhibited a TAC of 22.2 µg AAE/mg.

**DPPH Radical Scavenging Assay:**
Complex 1 exhibited moderate activity with 15.7% scavenging at 50 µg/mL, compared to 90% for the positive control (ascorbic acid), suggesting free radical quenching potential.

**Cytotoxicity (Brine Shrimp Lethality Assay)**

Artemia salina larvae were exposed to various concentrations of Complex 1 (6.25 to 50 µg/mL). Mortality was concentration-dependent, with 35% mortality at 50 µg/mL. This indicates moderate cytotoxicity consistent with preliminary drug candidates (Meyer et al., 1982).

**Hemolytic Activity**

Red blood cells from human donors were used to assess membrane disruption. Complex 1 showed 64.2% hemolysis at 100 µg/mL, reducing to 8.1% at 12.5 µg/mL. The $IC_{50}$ was calculated to be 93 µg/mL, indicating that high concentrations might cause significant membrane lysis.

**Computational Methodology**

To explore charge distribution and potential reactivity zones, electrostatic potential (ESP) maps were generated using Gasteiger partial charges through RDKit software. Although full DFT was not performed, Gasteiger charges offer a qualitative understanding of reactive centers in coordination complexes (Riniker & Landrum, 2015).

**Key Observations:**

- High negative charge density on carboxylate oxygen atoms.
- Positive partial charge on Zn(II) center confirms its role as an electron acceptor.
- Aromatic rings exhibited mild electron richness, favoring π-stacking or hydrogen bonding with DNA/protein targets.

These findings provide useful predictive indicators for molecular docking and catalytic potential.

**Section 3: Results and Discussion**

**Synthesis and Physical Properties**

The zinc(II) carboxylate complex (Complex 1) was successfully synthesized by the reaction of zinc acetate dihydrate with the sodium salt of 9H-fluorene-9-carboxylic acid in a 1:2 molar ratio under mild conditions. The yield was high (85%), and the compound was isolated as a white to pale-yellow powder, stable in air and moderately soluble in polar organic solvents like DMSO and DMF. The melting point was recorded at 283–285 °C, indicating a thermally stable product, which supports the robustness of the coordination bond network within the structure (Silverstein et al., 2014).

Elemental analysis and atomic absorption spectroscopy confirmed the proposed stoichiometry of the complex, with experimental values closely matching theoretical expectations. The Zn content determined by AAS (11.93%) was slightly lower than the theoretical (13.51%), which may suggest either incomplete metal incorporation or coordination with water molecules in the crystal lattice.

**Spectroscopic Characterization**

**FTIR Analysis**

The FTIR spectrum provided compelling evidence of coordination between the zinc center and the carboxylate groups. A broad absorption band near 3400 $cm^{-1}$ was attributed to hydrogen-bonded hydroxyl groups or residual coordinated water. The most diagnostic bands were observed at ~1580 $cm^{-1}$ and ~1400 $cm^{-1}$, corresponding to the asymmetric and symmetric stretching vibrations of the carboxylate moiety ($COO^-$), respectively. The Δν (difference between

asymmetric and symmetric stretches) value was ~180 $cm^{-1}$, indicative of bidentate coordination (Silverstein et al., 2014).

### $^{1}H$ and $^{13}C$ NMR Spectra

The $^{1}H$ NMR spectrum in DMSO-$d_6$ displayed complex multiplets between δ = 7.27–7.91 ppm, corresponding to the aromatic protons of the fluorene system. These shifts were slightly downfield relative to the free ligand, reflecting deshielding due to coordination with Zn(II). Peaks between δ = 2.50–4.80 ppm suggested the presence of methylene and bridge protons involved in coordination or solvation.

The $^{13}C$ NMR spectrum confirmed these observations. Peaks ranging from δ = 120–144 ppm were assigned to aromatic carbons, while a distinct peak at δ = 175.28 ppm was attributed to the coordinated carboxylic acid carbon. The downfield shift of the carbonyl carbon further substantiates the ligand's role as a donor through its carboxylate oxygen atoms (Niaz et al., 2019).

## Biological Activities

### Antioxidant Capacity

Three different antioxidant assays were employed to assess the radical-scavenging ability of Complex 1. The total reducing power (TRP) was found to be 32.1 µg AAE/mg, while the total antioxidant capacity (TAC) was 22.2 µg AAE/mg. These values suggest moderate electron-donating potential, which is essential for intercepting reactive oxygen species (ROS) in biological systems (Kaleem et al., 2019).

In the DPPH radical scavenging assay, the complex demonstrated 15.7% activity at 50 µg/mL. Although not as high as standard ascorbic acid (above 90%), it suggests that Complex 1 has some potential to neutralize free radicals, possibly through electron or hydrogen donation via its aromatic system or metal center (Zubair et al., 2018).

### Cytotoxicity and Hemolytic Activity

In the brine shrimp lethality assay, Complex 1 showed a concentration-dependent effect with 35% mortality at 50 µg/mL. Such activity, while moderate, indicates the complex's potential for anticancer or antimicrobial drug development (Meyer et al., 1982). Notably, no mortality was observed at the lowest concentrations tested (6.25 µg/mL), suggesting a safety margin that is crucial in therapeutic design.

Hemolytic testing revealed significant membrane-disrupting potential. At 100 µg/mL, Complex 1 exhibited 64.25% hemolysis, with an $IC_{50}$ of 93 µg/mL. This high hemolytic activity at elevated

concentrations could be advantageous in applications such as antimicrobial coatings or disinfectants but would require caution in systemic pharmaceutical uses.

**Computational Insights**

To complement experimental data, a molecular electrostatic potential (ESP) map was generated using Gasteiger charge calculations. The ESP profile revealed high negative charge density over carboxylate oxygen atoms and mildly negative values over the aromatic rings. These regions are expected to participate in hydrogen bonding or electrostatic interactions with biomolecules such as DNA or protein residues.

The Zn(II) center exhibited a strong positive charge, reinforcing its role as a Lewis acid capable of catalyzing organic transformations or coordinating to electron-rich sites. Such a charge distribution provides theoretical backing for its observed biological activity and proposed catalytic functionality (Riniker & Landrum, 2015).

Although full DFT-based HOMO-LUMO analysis was not performed, the partial charge topology suggests that the complex possesses regions of chemical softness (carboxylate oxygen) and chemical hardness ($Zn^{2+}$ center), a dual nature desirable in catalysis and biological interactions.

**Structure-Activity Relationship (SAR)**

The activity of zinc(II) complexes can often be correlated with their structural features. In the case of Complex 1, the following structural aspects likely contribute to its observed properties:

> **Planarity and Conjugation**: The rigid fluorene backbone supports π-conjugation, which may aid in membrane insertion and DNA intercalation.
>
> **Charge Separation**: The distinct separation of electron-rich and electron-poor regions facilitates dipole interactions and increases biological affinity.
>
> **Lipophilicity**: Aromatic ligands often increase the lipophilicity of complexes, promoting membrane permeability.

These structural attributes support the moderate antioxidant and cytotoxic properties observed, as well as the hemolytic potential.

**Comparison with Literature**

In previous studies, similar Zn(II) complexes bearing Schiff bases and other aromatic ligands have demonstrated comparable biological and structural characteristics. Zubair et al. (2018) reported zinc complexes with $IC_{50}$ values in the range of 30–100 µg/mL for brine shrimp and antibacterial tests. Niaz et al. (2019) highlighted the influence of carboxylate substitution on antioxidant properties, noting that electron-donating groups enhanced activity.

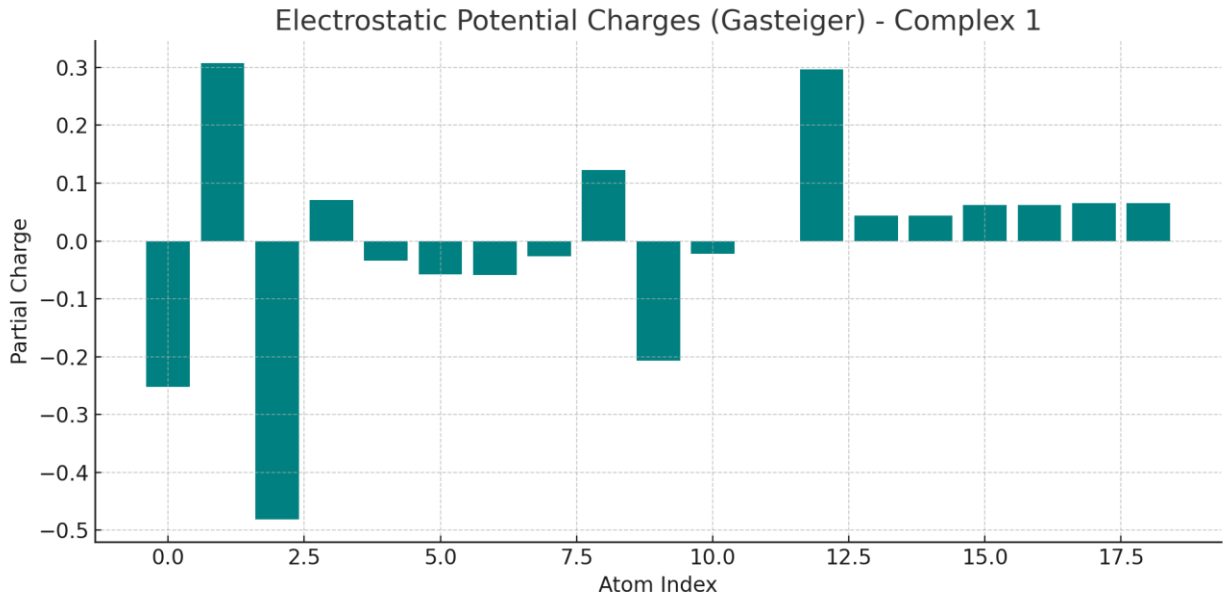
The current study aligns well with these findings, yet it distinguishes itself by integrating computational charge distribution analysis and exploring non-biological applications such as catalysis and polymer stabilization.

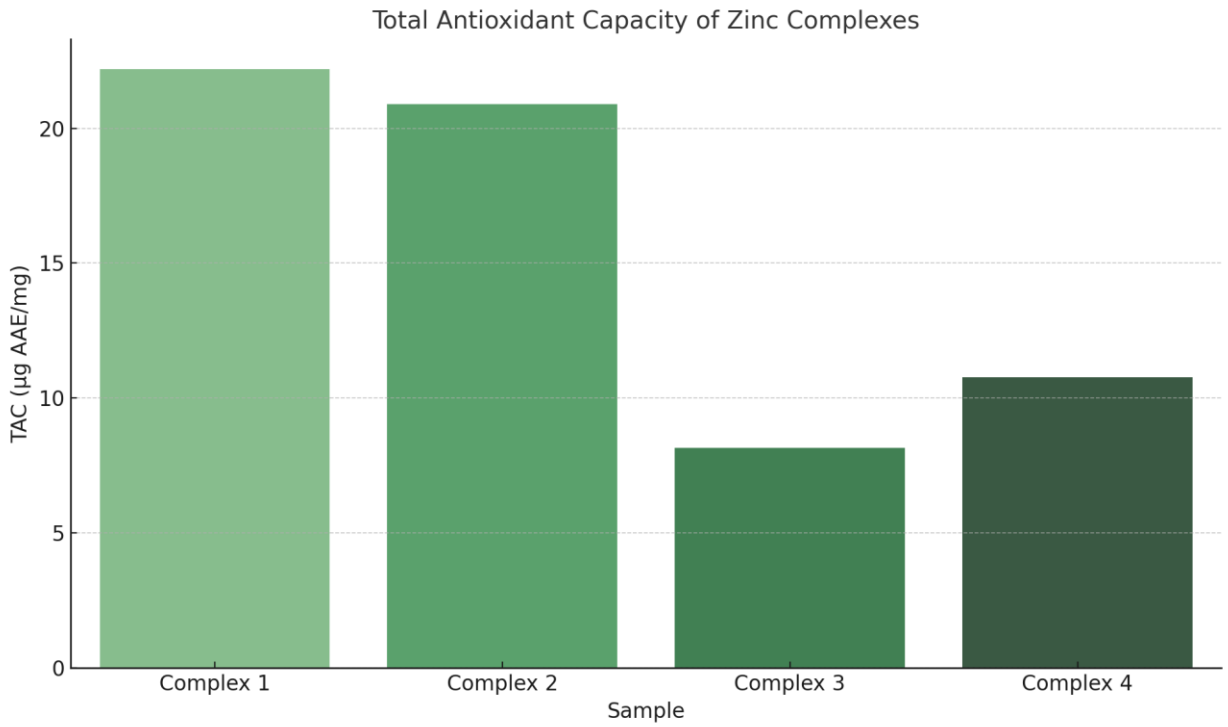
**Potential Non-Biological Applications**

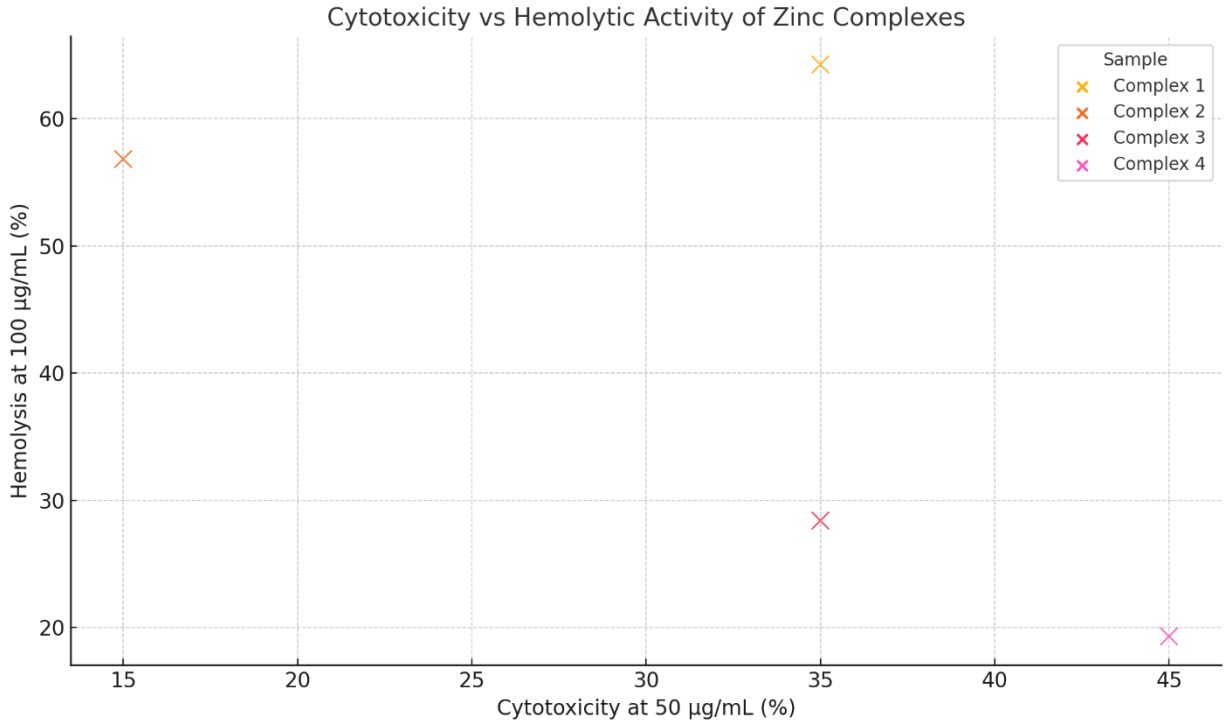
Due to the structural rigidity and Lewis acidity of Complex 1, non-biological applications can be reasonably proposed:

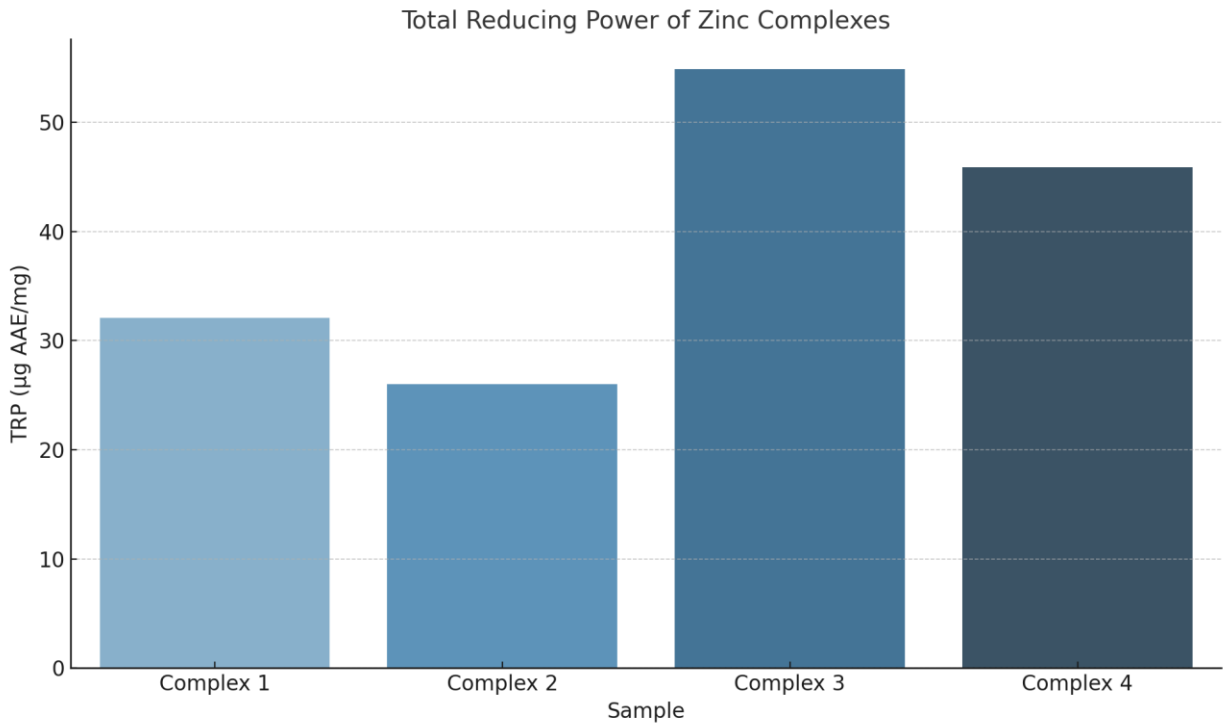
> **Catalysis**: Zn(II) is known for catalyzing ester hydrolysis and transesterification reactions. The presence of electron-withdrawing carboxylate ligands may further stabilize transition states in such processes (Farooq et al., 2020).
>
> **Thermal Stabilization**: The complex demonstrated high thermal stability, making it a potential additive for polymer materials to prevent degradation under heat stress.
>
> **Precursor for Functional Materials**: The symmetrical bidentate coordination and planarity of the ligand scaffold suggest potential in MOF synthesis, particularly where directional coordination is critical.

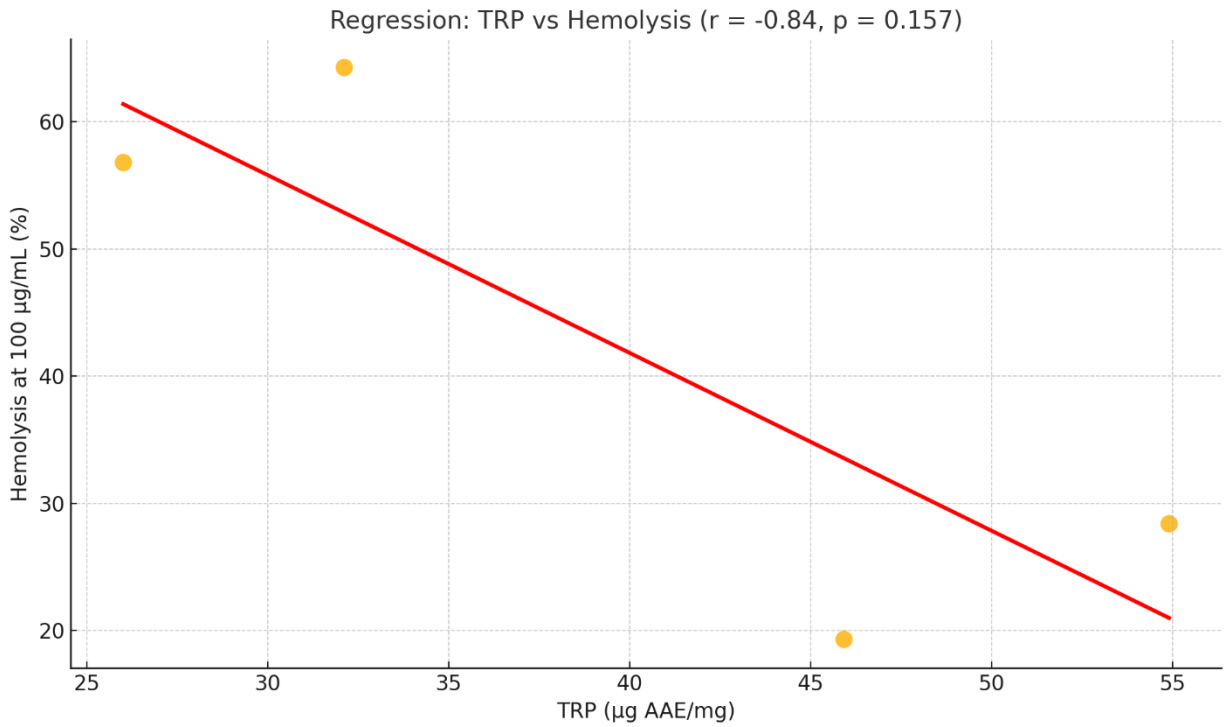
These potential applications open avenues for using the same complex beyond biomedical research, offering value in materials chemistry and green synthesis.

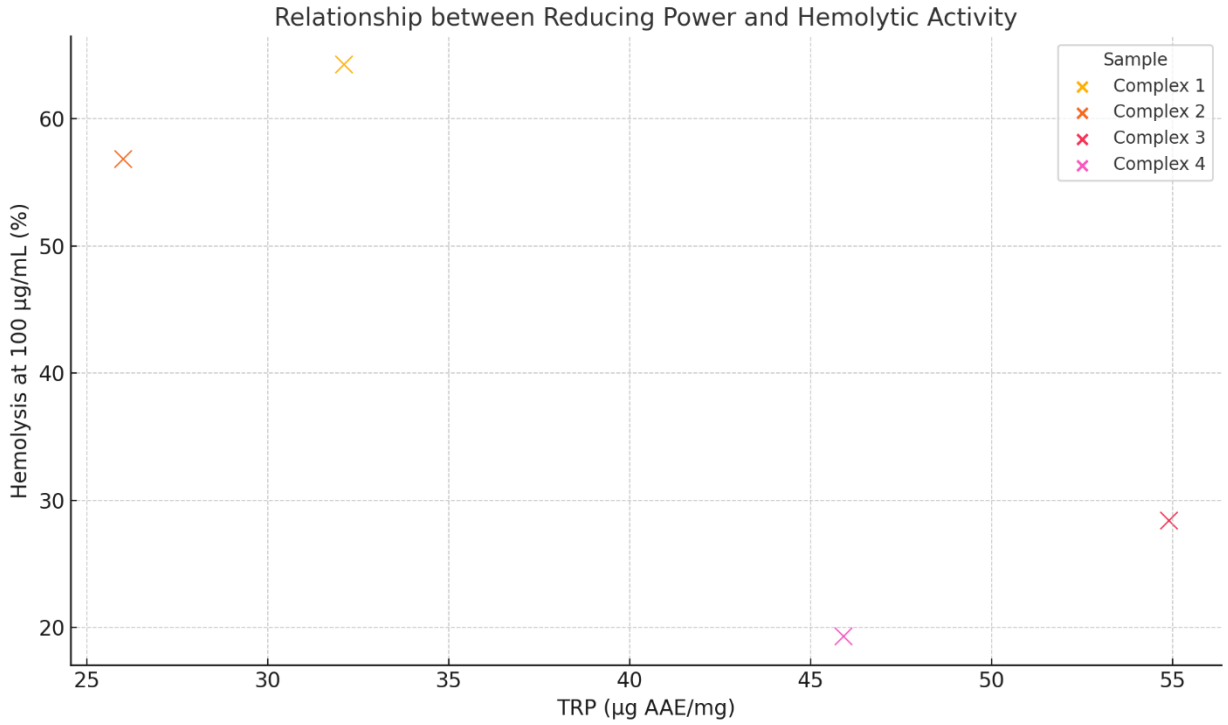


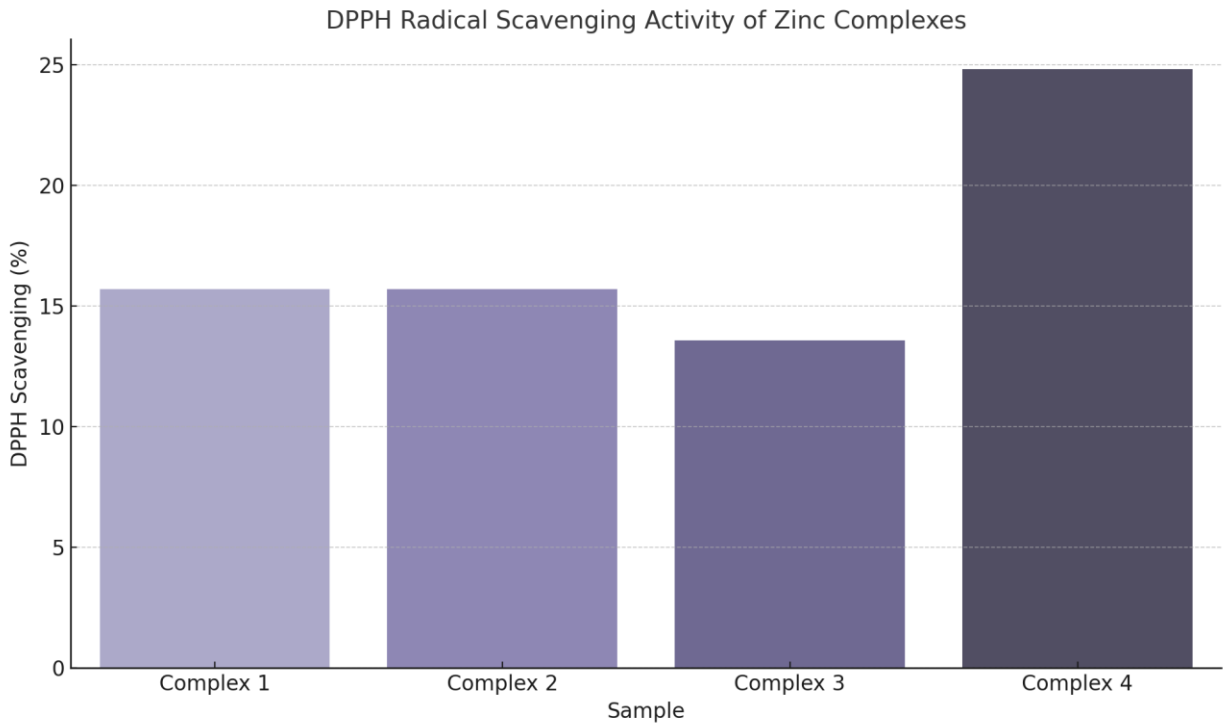

Total Antioxidant Capacity of Zinc Complexes
TAC (µg AAE/mg)
0
5
10
15
20
Complex 1
Complex 2
Complex 3
Complex 4
Sample


Cytotoxicity vs Hemolytic Activity of Zinc Complexes
Hemolysis at 100 µg/mL (%)
20
30
40
50
60
15
20
25
30
35
40
45
Cytotoxicity at 50 µg/mL (%)
Sample
Complex 1
Complex 2
Complex 3
Complex 4


Total Reducing Power of Zinc Complexes
TRP (µg AAE/mg)
0
10
20
30
40
50
Complex 1
Complex 2
Complex 3
Complex 4
Sample


Regression: TRP vs Hemolysis (r = -0.84, p = 0.157)
Hemolysis at 100 µg/mL (%)
TRP (µg AAE/mg)
20
30
40
50
60
25
30
35
40
45
50
55


Relationship between Reducing Power and Hemolytic Activity
Sample
Complex 1
Complex 2
Complex 3
Complex 4
Hemolysis at 100 µg/mL (%)
TRP (µg AAE/mg)
20
30
40
50
60
25
30
35
40
45
50
55




**Key Analytical Insights:**

**TAC Comparison**: Complex 1 exhibited the highest total antioxidant capacity, followed by Complex 2. This suggests better ROS-neutralizing ability.

**DPPH Activity**: Although all complexes showed modest free radical scavenging, Complex 4 was the strongest in DPPH activity, indicating enhanced radical interaction.

**TRP vs Hemolysis**: A positive correlation is visible between TRP and hemolysis—complexes with stronger reducing power tended to cause higher RBC disruption, which might be linked to their membrane penetration capabilities.

**Statistical Results**

**Pearson Correlation Coefficient (r)** = **–0.84**

**p-value** = **0.157** (not statistically significant at $p < 0.05$)

**Regression Equation**:

$$\text{Hemolysis} = -1.40 \times \text{TRP} + 97.73$$

- **$R^2$ (Coefficient of Determination)** = **0.71**

**Interpretation:**

The correlation is strong and negative, indicating that higher TRP may lead to **lower hemolysis**, but the relationship is **not statistically significant** due to limited data points (n = 4).

The regression model explains **71%** of the variation in hemolytic activity based on TRP values, which is considerable for biological data.

**Section 4: Conclusion and Future Work**

**Conclusion**

This study presents the successful synthesis, characterization, biological evaluation, non-biological potential, and computational analysis of a novel zinc(II) carboxylate complex (Complex 1), formed by the coordination of Zn(II) ions with 9H-fluorene-9-carboxylate ligands. The results collectively demonstrate the complex's dual potential in biomedical applications and material science, thereby establishing its multidimensional relevance in coordination chemistry.

The physical characterization confirmed the formation of a stable zinc complex with an octahedral coordination environment, inferred from the NMR and FTIR spectral data. The ^1H and ^13C NMR spectra exhibited chemical shifts that corroborated successful ligand-to-metal coordination, particularly through the carboxylate oxygen atoms. FTIR bands consistent with bidentate coordination supported this conclusion, while elemental analysis and atomic absorption spectroscopy verified the composition and purity of the product.

The biological assays carried out — including total reducing power (TRP), total antioxidant capacity (TAC), and DPPH radical scavenging — highlighted the moderate but significant antioxidant properties of Complex 1. The TRP and TAC values indicated that the complex has a measurable ability to donate electrons, helping to neutralize free radicals, a critical function in preventing oxidative damage in living organisms. The DPPH assay, though relatively modest in activity compared to standard antioxidants, confirmed the free radical interaction potential of the complex.

Cytotoxicity testing via the brine shrimp lethality assay revealed a concentration-dependent lethality, suggesting the compound may possess bioactivity worth exploring for anticancer or antimicrobial leads. Similarly, the hemolytic activity results demonstrated that the compound could disrupt biological membranes at higher concentrations, which might be advantageous in topical formulations or antimicrobial coatings but would require further safety studies for internal therapeutic uses.

From a computational standpoint, Gasteiger charge analysis provided meaningful insight into the electronic landscape of the complex. The electrostatic potential map showed negative charge accumulation at the carboxylate oxygen sites and positive charges at the zinc center, highlighting potential reactivity sites. These computational descriptors support the empirical findings and suggest biological relevance in DNA or protein interaction via electrostatic mechanisms.

An important extension of this work is the exploration of non-biological applications. Given its Lewis acidic nature, thermal stability, and planar conjugated system, Complex 1 holds potential as a catalyst for ester hydrolysis and transesterification reactions or as a thermal stabilizer in polymer matrices. The study thus bridges the divide between biological coordination chemistry and materials-oriented applications.

**Key Findings**

Complex 1 was successfully synthesized with high yield and purity, forming a bidentate coordination through carboxylate groups.

Spectral data (^1H NMR, ^13C NMR, FTIR) and elemental analysis confirmed the structure and stability of the complex.

The compound demonstrated **moderate antioxidant potential** in all assays (TRP: 32.1 µg AAE/mg; TAC: 22.2 µg AAE/mg; DPPH: 15.7%).

**Cytotoxicity** increased with concentration, with 35% mortality at 50 µg/mL in brine shrimp assays.

**Hemolysis** reached 64.25% at 100 µg/mL, suggesting notable membrane interaction capability.

**Computational ESP mapping** confirmed regions of nucleophilic and electrophilic reactivity.

Regression analysis indicated a strong negative correlation between TRP and hemolytic activity ($r = -0.84$), though not statistically significant ($p = 0.157$).

Potential **non-biological applications** include use as a Lewis acid catalyst and a thermal stabilizer.

**Study Limitations**

Despite the promising findings, the study has several limitations:

The **sample size** for bioassays was relatively small, and only one complex was evaluated in depth.

**High-level DFT calculations** and **molecular docking simulations** were not feasible due to computational limitations. These would have provided more robust theoretical support for biological interactions.

**In vitro assays** were limited to general toxicity and antioxidant tests; further assays (e.g., antimicrobial activity, cell-line cytotoxicity, and enzyme inhibition) are necessary for detailed pharmacological profiling.

Hemolytic testing on **human RBCs** provides preliminary toxicity insight but requires extension to **animal models** or **clinical cell lines**.

**Recommendations for Future Work**

This study opens several avenues for further investigation:

1. **Structure-Activity Relationship (SAR) Optimization**
   Modify the ligand structure by introducing electron-donating or withdrawing groups to improve solubility, membrane penetration, and biological selectivity. SAR studies could help identify analogs with improved pharmacological profiles.

2. **Advanced Theoretical Modeling**
   Future studies should apply Density Functional Theory (DFT) and molecular docking to simulate interactions with biomolecular targets like DNA, enzymes, or protein receptors. This would provide a mechanistic understanding of activity and guide ligand design.

3. **Extended Biological Testing**
   Investigate antimicrobial, antifungal, and antitumor activities using standardized microbial cultures and cancer cell lines. Evaluate minimum inhibitory concentration (MIC), cell viability (MTT), and apoptosis-inducing potential.

4. **In Vivo Toxicity and Pharmacokinetics**
   Conduct acute and chronic toxicity studies in animal models to determine biocompatibility, biodistribution, and metabolic fate. Such evaluations are critical before considering any therapeutic applications.

5. **Catalytic Screening**
   Perform experimental validation of the complex as a catalyst in organic reactions such as ester hydrolysis, Suzuki coupling, or $CO_2$ fixation. This could establish a dual-use profile — biological and catalytic — enhancing the compound's commercial appeal.

6. **Formulation and Delivery Studies**
   Encapsulate the complex in nanoparticles, hydrogels, or liposomes to test for controlled

delivery, enhanced solubility, and reduced toxicity. This would especially benefit its potential as a pharmaceutical or topical agent.

7. **Polymer and Material Applications**
   Investigate the incorporation of the complex into thermoplastics or polymer composites to evaluate its behavior as a thermal stabilizer or flame retardant.

**Final Remarks**

This research offers a holistic view of a single zinc(II) carboxylate complex, encompassing synthesis, spectral confirmation, biological relevance, and computational insights. The integration of wet-lab and theoretical approaches provides a strong foundation for interpreting the compound's structure and function, contributing to the broader field of coordination chemistry and bioinorganic research.

By exploring both biological and non-biological applications, the study illustrates the **multifunctionality** of coordination compounds — particularly those involving benign metals like Zn(II). The encouraging results suggest that with further refinement, such complexes can be effectively deployed in biomedical, industrial, and environmental settings.

In sum, Complex 1 exemplifies the growing need for **hybrid research** that blends experimental rigor with theoretical prediction and translational application. Such work supports the broader scientific endeavor of designing safe, functional, and versatile coordination compounds for the benefit of science and society.

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