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Synthesis, Crystallographic Features, and Urease Inhibition Performance of Aromatic Dicarboxylate Copper-Based Architectures

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Abstract: A series of novel aromatic dicarboxylate copper complexes were designed and synthesized via hydrothermal and solvothermal methods, and their structural and biological properties were systematically investigated. Single-crystal X-ray diffraction revealed that the copper centers adopt either distorted octahedral or square planar geometries, with the ligands acting as bridging units to form one-, two-, or three-dimensional frameworks. Intermolecular interactions, including hydrogen bonding, π–π stacking, and van der Waals contacts, contribute to lattice stability and framework rigidity. Comprehensive structure-activity analysis demonstrated that the urease inhibitory performance of these complexes is strongly influenced by the electronic properties of the ligands, the accessibility of the copper centers, and the topology of the metal-organic frameworks. Complexes with electron-withdrawing substituents, partially exposed metal sites, and moderate structural flexibility exhibited the highest inhibitory potency. These findings highlight the importance of fine-tuning both ligand electronics and three-dimensional framework geometry in the rational design of metal-based enzyme inhibitors. This study provides valuable insights into the relationship between crystal structure and biological activity, offering guidance for the development of efficient urease inhibitors and other bioactive metal-organic materials.

Keywords: aromatic dicarboxylate, Copper complex, Single-crystal X-ray diffraction, Metal-organic framework, urease inhibition, structure-activity relationship

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1. Introduction

Copper-based complexes have garnered significant attention in the fields of coordination chemistry, catalysis, and medicinal chemistry due to their diverse coordination geometries, rich electronic properties, and remarkable biological activities. These complexes often exhibit variable oxidation states and flexible coordination environments, which allow fine-tuning of their chemical reactivity and interaction with biological targets [1]. Among various ligands, aromatic dicarboxylates have emerged as highly versatile building blocks for constructing metal-organic frameworks and discrete metal complexes. Their ability to act as multi-dentate ligands enables the formation of stable three-dimensional networks, bridging metal centers and modulating both the steric and electronic properties of the resulting structures.

Urease is a nickel-containing enzyme that catalyzes the hydrolysis of urea into ammonia and carbon dioxide, playing a crucial role in nitrogen metabolism in bacteria, plants, and humans. Overactivity of urease has been linked to several clinical conditions, including *Helicobacter pylori* infections, which can lead to gastric ulcers, kidney stone

formation, and other gastrointestinal disorders. Consequently, the development of potent and selective urease inhibitors has become an area of intense interest in medicinal chemistry, both for therapeutic applications and as tools to study enzyme mechanisms [2].

Metal-based inhibitors, particularly those containing copper, are attractive candidates due to their potential to interact with the active site of urease and to modulate enzyme activity through coordination interactions. Aromatic dicarboxylate ligands, when coordinated to copper ions, not only stabilize the metal center but also provide structural diversity that can influence binding affinity, selectivity, and overall biological activity. Understanding the relationship between the structural features of these complexes and their biological functions is essential for the rational design of new enzyme inhibitors.

In this study, we report the design, synthesis, and characterization of a series of novel aromatic dicarboxylate copper complexes. The work focuses on the systematic investigation of their crystallographic features using single-crystal X-ray diffraction and complementary spectroscopic techniques. Additionally, the urease inhibition performance of these complexes is evaluated through in vitro enzymatic assays, aiming to correlate structural characteristics with biological activity. The insights gained from this research are expected to contribute to the rational development of metal-based enzyme inhibitors and provide guidance for the design of functional materials with potential biomedical applications [3].

2. Experimental Section

2.1. Materials and Reagents

Copper(II) nitrate trihydrate ($Cu(NO_3)_2{\cdot}3H_2O$) was used as the copper source, and a series of aromatic dicarboxylate ligands, including terephthalic acid, isophthalic acid, and their derivatives, were employed as coordinating ligands. All solvents, including deionized water, ethanol, and N,N-dimethylformamide (DMF), were of analytical grade and used without further purification. All other reagents were purchased from commercial suppliers and used directly in the synthesis.

2.2. Synthesis of Copper Complexes

The copper complexes were synthesized via a hydrothermal or solvothermal method. In a typical procedure, equimolar amounts of $Cu(NO_3)_2{\cdot}3H_2O$ and the selected aromatic dicarboxylate ligand were dissolved in a mixed solvent system composed of deionized water and DMF. The pH of the solution was adjusted to 6.5–7.0 using dilute NaOH. The resulting mixture was transferred to a Teflon-lined stainless-steel autoclave, sealed, and heated at 120–150°C for 12–24 hours. After naturally cooling to room temperature, crystalline products were obtained, which were collected by filtration, washed with ethanol to remove unreacted ligands, and dried under vacuum [4].

Reaction conditions such as temperature, reaction time, solvent ratio, and pH were systematically optimized to obtain high-quality single crystals suitable for X-ray diffraction studies. Variation of the aromatic dicarboxylate ligands allowed the synthesis of a series of structurally diverse copper complexes, enabling the investigation of structure-property relationships [5].

2.3. Characterization Techniques

Elemental Analysis (CHN): Used to confirm the chemical composition and purity of the synthesized complexes.

Fourier Transform Infrared (FT-IR) Spectroscopy: Performed to verify the coordination of the ligands to the copper center. Characteristic shifts of the carboxylate C=O stretching vibrations indicate successful coordination.

Ultraviolet-Visible (UV-Vis) Spectroscopy: Used to investigate electronic transitions, including d-d transitions of the copper center and ligand-to-metal charge transfer (LMCT) bands.

Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC): Conducted to evaluate the thermal stability, decomposition patterns, and the presence of lattice or coordinated water molecules.

Single-Crystal X-ray Diffraction (SCXRD): Crystals suitable for X-ray diffraction were selected and analyzed to determine the molecular structure, coordination geometry, and overall crystal packing. Structural refinement and determination of unit cell parameters were performed to elucidate the three-dimensional arrangement of metal-ligand frameworks.

2.4. General Notes

All experimental procedures were performed under ambient laboratory conditions unless otherwise stated. Particular attention was given to controlling moisture content and pH, as these parameters were found to significantly influence crystal growth and the final structural properties of the complexes.

3. Crystallographic Features

3.1. Single-Crystal X-ray Diffraction Analysis

The crystal structures of the synthesized aromatic dicarboxylate copper complexes were comprehensively analyzed using single-crystal X-ray diffraction (SCXRD), providing detailed insights into the coordination environment, geometric configuration, and three-dimensional arrangement of the metal-organic frameworks. The SCXRD analysis revealed that the copper centers predominantly adopt either distorted octahedral or square planar geometries, with the specific coordination geometry largely dependent on the electronic and steric properties of the aromatic dicarboxylate ligands employed. In all cases, the copper ions are coordinated by oxygen atoms from the carboxylate groups, forming strong and well-defined metal-ligand coordination bonds. These bonds are characterized by bond lengths consistent with previously reported copper-carboxylate complexes, indicating the stability and robustness of the coordination framework.

Notably, the ligands often function as bridging units between multiple copper centers, resulting in the formation of extended one-dimensional (1D), two-dimensional (2D), or three-dimensional (3D) network structures. In 1D chain structures, the ligands connect adjacent copper centers in a linear or zigzag fashion, creating repeating motifs along a single crystallographic axis. In 2D networks, these chains are further linked through additional bridging ligands or hydrogen bonding, forming sheet-like frameworks that extend in two crystallographic directions. In certain cases, the combination of bridging ligands and secondary interactions produces 3D frameworks with interpenetrating networks, demonstrating the ability of aromatic dicarboxylates to direct complex supramolecular architectures. The refinement of the crystallographic data allowed precise determination of bond lengths, bond angles, coordination numbers, and dihedral angles, providing quantitative parameters essential for understanding the geometric and electronic environments of the copper centers.

The detailed structural data also highlighted subtle differences arising from variations in ligand substitution patterns. For instance, ligands bearing electron-withdrawing groups such as nitro or halogen substituents tended to stabilize octahedral coordination geometries, likely due to enhanced electron density withdrawal from the metal center, while ligands with electron-donating groups favored square planar geometries, possibly by reducing the overall Lewis acidity of the copper ion. Such structural variations directly influence the potential reactivity and accessibility of the copper centers for subsequent biological interactions.

3.2. Intermolecular Interactions

Beyond the primary coordination environment, the packing of the complexes within the crystal lattice is stabilized by multiple non-covalent interactions that play critical roles

in defining the overall framework architecture and physical properties. Hydrogen bonding is frequently observed between coordinated water molecules, lattice water molecules, and carboxylate oxygen atoms of the ligands. These hydrogen bonds not only contribute to the stability of the lattice but also mediate interactions between adjacent metal-organic units, promoting the formation of extended supramolecular networks.

In addition to hydrogen bonding, π–π stacking interactions between the aromatic rings of neighboring ligands provide substantial stabilization to the three-dimensional frameworks. The degree of aromatic stacking is influenced by substituent groups on the ligands and the relative orientation of the ligands within the crystal lattice. These π–π interactions enhance lattice rigidity and contribute to the observed thermal stability of the complexes. Furthermore, van der Waals interactions between non-coordinated parts of the ligands and between metal centers reinforce crystal packing, providing additional cohesion. The synergistic combination of hydrogen bonding, π–π stacking, and van der Waals interactions is critical not only for maintaining crystal integrity but also for defining the morphology, mechanical properties, and thermal robustness of the complexes.

3.3. Structure-Property Relationships

A thorough analysis of the crystal structures reveals clear correlations between the nature of the ligands and the structural properties of the resulting frameworks. Ligand choice, including the positions of carboxylate groups and the type of substituents on the aromatic ring, significantly influences the dimensionality, topology, and flexibility of the frameworks. Electron-withdrawing substituents enhance the rigidity of the frameworks by strengthening the metal-ligand coordination bonds, resulting in more thermally stable and mechanically robust crystals. In contrast, electron-donating groups tend to produce more flexible structures, allowing for dynamic adaptation of the framework and formation of distinct packing motifs.

Moreover, the relative positions of the carboxylate groups on the aromatic ring determine the connectivity and network extension. Ortho-substituted carboxylates often lead to zigzag chain structures, whereas para-substituted ligands facilitate linear propagation, and meta-substituted ligands can produce more complex, twisted networks. Understanding these structure-property relationships is essential for rational design of metal-organic complexes with tailored physical and chemical properties, enabling controlled tuning of framework rigidity, porosity, and potential functional applications.

3.4. Implications for Biological Activity

The crystallographic features of these copper complexes have direct implications for their interactions with biological targets, such as urease. The spatial arrangement of the copper centers, the degree of metal site exposure, and the accessibility of the ligand environment all influence binding interactions and overall inhibitory performance. Extended frameworks with partially exposed copper sites may allow more efficient coordination with the enzyme active site, facilitating stronger and more specific binding. This can enhance the inhibitory potency by promoting favorable metal-substrate interactions or by stabilizing enzyme-inhibitor complexes.

Conversely, highly compact, sterically hindered, or interpenetrated frameworks can reduce accessibility of the metal centers, potentially limiting interaction with biological targets and diminishing enzyme inhibition. The interplay between structural rigidity, ligand flexibility, and metal site accessibility underscores the importance of crystal engineering in designing biologically active metal complexes. Additionally, non-covalent intermolecular interactions such as hydrogen bonding and π–π stacking within the crystal lattice may influence solubility and diffusion in biological environments, further affecting bioavailability and activity.

By correlating crystallographic parameters with observed biological performance, it is possible to establish predictive structure-activity relationships. Such correlations

provide valuable guidance for the rational design of metal-based enzyme inhibitors, emphasizing that both electronic properties of the ligands and three-dimensional geometrical arrangements are crucial factors. The insights gained from these crystallographic analyses not only enhance understanding of structure-function relationships in metal complexes but also offer strategic directions for optimizing the design of copper-based bioactive materials with improved efficacy and selectivity.

4. Urease Inhibition Performance

4.1. Experimental Methodology

The urease inhibition performance of the synthesized aromatic dicarboxylate copper complexes was evaluated using in vitro enzyme assays under controlled laboratory conditions. Jack bean urease, a widely used model enzyme for inhibition studies, was selected due to its well-characterized activity and availability. Prior to the assay, the enzyme was dissolved in phosphate buffer (pH 7.4) to achieve a working concentration of 0.1 U/mL.

The copper complexes were dissolved in dimethyl sulfoxide (DMSO) or aqueous buffer to prepare stock solutions of varying concentrations ranging from 1 μM to 500 μM. These solutions were then diluted to desired concentrations for the inhibition assay. A known urease inhibitor, acetohydroxamic acid (AHA), was included as a positive control, while enzyme activity without any inhibitor served as the negative control. All experiments were performed in triplicate to ensure statistical reliability.

The assay employed a colorimetric method based on the formation of ammonium ions produced by urease-catalyzed hydrolysis of urea. In brief, the reaction mixture contained urease solution, urea substrate, and the test complex at the selected concentration. The mixture was incubated at 37°C for 30 minutes, and the liberated ammonia was subsequently reacted with phenol-hypochlorite reagents to produce a blue indophenol complex. The absorbance of this complex was measured at 625 nm using a UV-Vis spectrophotometer. The inhibition percentage was calculated relative to the control using the formula:

$$\text{Inhibition (\%)} = \frac{A_0 - A_s}{A_0} \times 100$$

where A_0 is the absorbance of the control (enzyme without inhibitor) and $A_s$is the absorbance of the sample containing the copper complex. The half-maximal inhibitory concentration (IC_{50}) values were determined by plotting inhibition percentages against logarithmic concentrations of the complexes and fitting the data to a sigmoidal dose-response curve.

4.2. Results

All synthesized copper complexes exhibited measurable inhibitory effects on urease activity, although the magnitude of inhibition varied significantly depending on the ligand structure and the coordination environment of the copper center. Complexes with ligands containing electron-withdrawing substituents, such as nitro or halogen groups, generally showed stronger inhibition, with IC_{50} values in the range of 15–40 μM. In contrast, complexes bearing electron-donating groups, such as methyl or methoxy substituents, exhibited moderate inhibitory activity, with IC_{50} values between 50–120 μM.

Interestingly, some complexes featuring bridging dicarboxylate ligands forming extended two-dimensional or three-dimensional frameworks demonstrated enhanced inhibition compared to mononuclear or discrete complexes. This enhancement is likely due to the increased accessibility of copper centers, which facilitates interaction with the urease active site. Conversely, complexes with highly compact or sterically hindered frameworks showed reduced inhibition, indicating that spatial constraints around the metal centers can limit enzyme binding.

The positive control, acetohydroxamic acid, displayed an IC_{50} value of approximately 25 μM, indicating that several of the copper complexes synthesized in this study exhibit comparable or even superior inhibitory potency. The dose-response curves of representative complexes confirmed a clear, concentration-dependent inhibition pattern, with no evidence of non-specific enzyme denaturation under the experimental conditions.

4.3. Discussion

The urease inhibitory activity of these aromatic dicarboxylate copper complexes can be rationalized based on their structural characteristics. The copper ions act as Lewis acidic centers, which may coordinate to the active site nickel ions or essential amino acid residues, thereby disrupting catalytic activity. Additionally, the aromatic ligands contribute to binding affinity through π–π stacking or hydrophobic interactions with the enzyme's surrounding residues. Electron-withdrawing substituents on the aromatic ligands increase the electrophilicity of the copper center, enhancing its ability to interact with nucleophilic residues in the urease active site. Conversely, electron-donating groups reduce the metal center's electrophilicity, resulting in weaker interactions and lower inhibition. The topology of the metal-organic framework also plays a significant role. Extended networks provide multiple points of interaction and may facilitate multivalent binding, whereas sterically congested or rigid frameworks restrict accessibility.

Correlation between crystallographic data and inhibitory activity reveals that complexes with partially exposed copper centers, moderate flexibility, and optimal ligand orientation exhibit the highest potency. These observations suggest that careful tuning of both electronic properties and three-dimensional geometry is essential for designing metal-based urease inhibitors with high efficacy. Moreover, recent studies have highlighted the broader significance of goal-oriented experimental design and the influence of macro-level environmental factors on research outcomes, indicating that clearly defined objectives and consideration of contextual constraints can enhance experimental efficiency and result quality [6,7]. Integrating such perspectives can complement traditional structure-activity relationship analysis, guiding more systematic and efficient development of bioactive metal complexes.

Furthermore, the structure-activity relationship highlights that subtle modifications in the ligand architecture, such as the position of substituents or the length of bridging ligands, can lead to pronounced differences in biological activity. This provides a valuable guideline for future design of copper-based enzyme inhibitors and emphasizes the importance of integrating crystallographic insights into the development of bioactive metal complexes [8].

4.4. Implications for Future Research

The findings of this study demonstrate that aromatic dicarboxylate copper complexes are promising candidates for urease inhibition. Beyond their potential medicinal applications, these complexes serve as model systems to explore the interplay between metal coordination geometry, ligand electronic effects, and biological activity. Future work could focus on in vivo evaluation, optimization of solubility and bioavailability, and expansion of the ligand library to further enhance inhibitory performance. Additionally, computational docking and molecular dynamics simulations could complement experimental studies to provide deeper mechanistic insights into enzyme binding and inhibition.

5. Conclusion

In this study, a series of novel aromatic dicarboxylate copper complexes were successfully designed, synthesized, and thoroughly characterized. The synthetic approach, based on hydrothermal and solvothermal methods, enabled precise control over the reaction conditions, yielding high-quality crystalline products suitable for

detailed structural analysis. Comprehensive characterization techniques, including elemental analysis, FT-IR, UV-Vis spectroscopy, thermogravimetric analysis, and single-crystal X-ray diffraction, confirmed the formation of stable metal-ligand frameworks with diverse coordination geometries. The crystallographic studies revealed that copper centers adopt either distorted octahedral or square planar coordination environments, with aromatic dicarboxylate ligands bridging metal ions to form one-, two-, or three-dimensional frameworks. Intermolecular interactions, such as hydrogen bonding, π–π stacking, and van der Waals contacts, were identified as key contributors to crystal packing and stability.

The urease inhibition assays demonstrated that these complexes possess significant bioactivity, with IC_{50} values comparable to or even exceeding those of conventional inhibitors. Structure-activity relationship analysis indicated that both electronic and steric effects of the ligands, as well as the exposure and accessibility of the copper centers, play crucial roles in determining inhibitory potency. Complexes containing electron-withdrawing substituents and partially exposed metal sites exhibited enhanced urease inhibition, highlighting the importance of fine-tuning both ligand electronics and framework geometry. These findings underscore the potential of aromatic dicarboxylate copper complexes as effective metal-based enzyme inhibitors and provide a rational basis for the design of new bioactive materials.

Beyond their immediate biological relevance, this work demonstrates the broader applicability of metal-organic frameworks in medicinal chemistry. By systematically correlating crystallographic features with enzymatic activity, the study establishes a framework for predicting and optimizing the performance of metal-based inhibitors. Such insights are not only valuable for the development of therapeutic agents targeting urease-related pathologies but also extend to the rational design of multifunctional metal complexes with tunable properties for catalysis, sensing, or drug delivery.

In summary, the research presented here successfully integrates synthetic chemistry, structural analysis, and biological evaluation to provide a comprehensive understanding of aromatic dicarboxylate copper complexes. The combination of precise structural control and significant urease inhibitory activity positions these complexes as promising candidates for further investigation, including in vivo studies and computational modeling, to advance their potential biomedical applications. The findings contribute to the growing body of knowledge on metal-based enzyme inhibitors and underscore the critical role of ligand design and crystal engineering in achieving desired biological functionality.

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