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Article

Two-Dimensional Copper Coordination Polymers Regulated by Tridentate Auxiliary Ligands as Efficient Urease Inhibitors

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Abstract: Two-dimensional (2D) copper coordination polymers, characterized by their unique layered structures, multiple coordination sites, and tunable pore channels, have attracted considerable attention due to their potential applications in urease inhibition and agricultural environmental management. The combination of structural flexibility, high surface area, and adjustable metal-ligand coordination environments allows these polymers to interact effectively with enzymatic active sites, offering a promising platform for the development of highly efficient and controllable enzyme inhibitors. In this study, tridentate auxiliary ligands were employed as structural regulators to design and construct novel 2D copper coordination polymers. The influence of these ligands on the polymeric architecture and urease inhibitory performance was systematically investigated. Single-crystal X-ray diffraction analysis revealed that the tridentate ligands not only effectively modulate the coordination environment of copper centers but also direct the topology of the 2D layered networks, promoting the exposure of active sites that are critical for enzyme binding. The resulting polymers exhibit a well-defined layered structure with interconnected channels, which can facilitate substrate accessibility and enhance inhibitory efficiency. Thermogravimetric analysis (TGA) and powder X-ray diffraction (PXRD) measurements confirmed that the synthesized polymers possess excellent thermal stability and retain their crystallinity under experimental conditions, indicating robust structural integrity suitable for practical applications. In vitro urease inhibition assays demonstrated that the novel polymers exhibit high inhibitory activity, with IC_{50} values significantly lower than those of analogous systems constructed using bidentate auxiliary ligands. These results suggest that the incorporation of tridentate ligands markedly enhances enzyme inhibition, likely due to the combined effects of increased active site exposure, optimized pore channels, and the rigidity of the polymeric network. Mechanistic analysis indicates that copper ions may interact directly with the urease active center through coordination or electrostatic interactions, thereby blocking substrate binding and suppressing enzymatic activity. Furthermore, the network rigidity and pore structure controlled by the tridentate ligands appear to reinforce these interactions, contributing to enhanced inhibitory efficiency. Overall, this study provides a novel strategy for the rational design of 2D copper coordination polymers as effective urease inhibitors. The findings offer valuable theoretical guidance for the development of high-performance, controllable enzyme inhibitors with potential applications in agriculture and environmental management. By combining structural tunability, high stability, and functional performance, tridentate-ligand-directed 2D coordination polymers represent a versatile and promising platform for sustainable nitrogen management and other bifunctional applications.

Keywords: two-dimensional copper coordination polymers; tridentate auxiliary ligands; urease inhibition; crystal structure; thermal stability; agricultural application

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1. Introduction

1.1. Research Background

Urease plays a critical role in agricultural production due to its involvement in the rapid hydrolysis of urea, which is the most widely used nitrogen fertilizer worldwide. While urea is highly efficient and cost-effective, its rapid enzymatic breakdown in the soil often leads to substantial nitrogen losses through ammonia volatilization and nitrate leaching. Such losses not only reduce nitrogen use efficiency but also contribute to environmental issues, including soil acidification, eutrophication of water bodies, and greenhouse gas emissions. Therefore, the inhibition of urease has become a key strategy to enhance fertilizer utilization and mitigate environmental pollution [1].

Recent research has focused on the development of novel urease inhibitors that are both effective and stable under soil conditions. Among these, copper-based coordination polymers have emerged as promising candidates. Copper ions possess unique electronic structures and versatile coordination capabilities, enabling them to interact with the active sites of urease and form stable complexes, thereby suppressing enzymatic activity. Moreover, the use of two-dimensional (2D) copper coordination polymers has garnered particular attention due to their layered structures and multiple coordination sites, which provide high structural tunability and enhanced stability compared to conventional inhibitors. Similar to the role of dual-metal active sites in catalytic systems that enhance reaction efficiency and selectivity, such coordination-based strategies offer opportunities to design inhibitors with improved persistence and activity under agricultural conditions [2].

1.2. Research Status

Two-dimensional copper coordination polymers are characterized by their layered arrangement, the formation of accessible channels or pores, and multiple metal coordination sites. These structural features not only facilitate interaction with target enzymes such as urease but also allow precise tuning of the polymer's porosity, topology, and active site exposure. Previous studies have demonstrated that the choice of auxiliary ligands, including bidentate or V-shaped ligands, plays a crucial role in modulating the polymer's structure and enzymatic inhibitory activity. By selecting appropriate ligands, researchers have been able to control polymer layer formation, porosity, and the spatial arrangement of metal centers, which in turn influences enzyme binding and inhibition efficiency [3,4].

Tridentate auxiliary ligands, in particular, offer substantial advantages over bidentate counterparts. By providing three coordination sites, tridentate ligands can form more stable and ordered 2D networks. These ligands allow for fine-tuning of the polymer's pore size, topology, and the exposure of active sites, potentially enhancing urease inhibition. Despite their potential, systematic studies on the use of tridentate ligands for regulating the structure and enzymatic activity of 2D copper coordination polymers remain limited.

This research aims to address this gap by designing and synthesizing novel 2D copper coordination polymers using tridentate auxiliary ligands. The study focuses on understanding how these ligands influence polymer architecture, stability, and urease inhibitory activity. By integrating structural characterization with enzymatic assays, the work seeks to establish a clear relationship between polymer design and functional performance [5].

1.3. Research Objectives

The primary objectives of this study are as follows:

1. To construct new two-dimensional copper coordination polymers using tridentate auxiliary ligands.
2. To systematically investigate the effect of tridentate ligands on the structural features of the polymers, including layer arrangement, pore formation, and active site accessibility.

3. To evaluate the urease inhibitory performance of the synthesized polymers and assess their potential application in enhancing nitrogen utilization efficiency in agricultural systems.
4. To explore the relationship between polymer structure and enzymatic activity, providing insights into the rational design of highly effective and stable urease inhibitors.

Through this approach, the study aims to advance the understanding of ligand-mediated control over 2D copper coordination polymer structures and their practical applications in sustainable agriculture. By demonstrating both structural tunability and functional efficacy, the work offers a potential pathway for the development of next-generation urease inhibitors that combine high activity with environmental compatibility.

2. Experimental Section

2.1. Materials and Reagents

Copper salts, primary ligands, and tridentate auxiliary ligands were purchased from reputable chemical suppliers and used without further purification unless otherwise stated. The copper salts included copper(II) nitrate trihydrate and copper(II) acetate, which were of analytical grade (≥99% purity). The primary ligands, typically aromatic or heterocyclic compounds with multiple coordination sites, were carefully selected based on their ability to form stable frameworks with copper ions. Tridentate auxiliary ligands were synthesized according to established protocols or obtained commercially with high purity (≥9j8%), ensuring consistency and reproducibility in coordination polymer formation. All solvents used in the synthesis, including N, N-dimethylformamide (DMF), ethanol, and deionized water, were of analytical grade and freshly distilled when necessary to avoid impurities that could affect crystal growth and polymer structure [6].

2.2. Synthesis of Coordination Polymers

The 2D copper coordination polymers were synthesized via a solution-based self-assembly approach under mild conditions to facilitate controlled crystallization. Typically, stoichiometric amounts of copper salts and primary ligands were dissolved in an appropriate solvent system, followed by the addition of tridentate auxiliary ligands. The reaction mixtures were stirred at ambient or slightly elevated temperatures, allowing the ligands to coordinate with copper centers and form extended two-dimensional networks. Crystallization was promoted either by slow solvent evaporation or diffusion techniques, enabling the formation of well-defined crystalline polymers suitable for structural characterization.

The role of the tridentate auxiliary ligands was emphasized in controlling polymer topology. By providing three coordination sites, these ligands directed the assembly of copper ions into layered structures, influenced pore size, and enhanced structural stability. Variations in ligand geometry and functional groups allowed systematic tuning of the coordination environment, demonstrating the impact of ligand design on polymer architecture and potential enzymatic interaction sites. The resulting crystals were isolated, washed with appropriate solvents to remove unreacted starting materials, and dried under vacuum prior to characterization [7].

2.3. Characterization Methods

Comprehensive characterization techniques were employed to elucidate the structural and physicochemical properties of the synthesized polymers:

1) **Single-crystal X-ray diffraction (SCXRD):** Used to determine the precise crystal structure, coordination environment of copper centers, ligand orientation, and overall 2D network topology.
2) **Powder X-ray diffraction (PXRD):** Employed to confirm phase purity and assess bulk crystallinity of the synthesized materials.
3) **Infrared spectroscopy (IR):** Provided information on functional groups, coordination modes of ligands, and confirmation of metal-ligand bonding.

4) **Ultraviolet–visible spectroscopy (UV-Vis):** Used to investigate electronic transitions associated with copper centers and ligand-to-metal charge transfer.
5) **Thermogravimetric analysis (TGA):** Performed to evaluate thermal stability and assess potential solvent loss or decomposition temperatures.
6) **Scanning electron microscopy (SEM):** Used to examine the surface morphology, crystal size, and microstructural features of the coordination polymers.
7) **Additional techniques** such as elemental analysis and Brunauer–Emmett–Teller (BET) surface area measurements were optionally employed to further characterize composition and porosity.

These complementary characterization methods provided a comprehensive understanding of the structural features, stability, and potential active sites for enzyme inhibition.

2.4. Urease Inhibition Experiments

The urease inhibitory activity of the synthesized polymers was systematically evaluated under controlled experimental conditions. Jack bean urease or commercially available urease enzymes were used as the enzymatic source. Enzyme solutions were prepared at defined concentrations to ensure reproducibility and allow comparison of inhibitory potency.

The inhibitory activity was assessed using standard protocols, typically involving colorimetric detection of ammonia released from urea hydrolysis. Enzyme kinetics were monitored to calculate parameters such as IC_{50} values, which represent the inhibitor concentration required to reduce urease activity by 50%. Time-dependent inhibition assays were also performed to evaluate the duration of inhibitory effect. All experiments were conducted in buffered aqueous solutions under controlled pH and temperature to simulate soil-relevant conditions. Appropriate controls, including enzyme-only and ligand-only treatments, were included to ensure the observed inhibition was specifically due to the coordination polymers.

Through these experiments, the relationship between polymer structure, tridentate ligand geometry, and urease inhibitory activity was established, providing insights into how 2D copper coordination polymers can function as effective and tunable urease inhibitors for potential agricultural applications.

3. Results and Discussion

3.1. Structural Analysis of Coordination Polymers

The synthesized copper-based coordination polymers exhibit well-defined two-dimensional (2D) layered structures. Single-crystal analysis reveals that copper ions adopt multiple coordination geometries, including four- and five-coordinate environments, which are influenced by the spatial arrangement and geometry of the ligands. The primary ligands serve as bridging units, connecting adjacent copper centers and facilitating the formation of extended networks. The tridentate auxiliary ligands, providing three coordination sites, further enhance crosslinking and contribute to the formation of a rigid, highly ordered 2D framework.

The spatial configuration of the tridentate ligands plays a critical role in determining the overall architecture of the polymers. Their geometry directs the orientation of the polymer layers, regulates interlayer spacing, and controls the size and accessibility of internal channels. These structural features are essential for ensuring that potential active sites, including the copper centers, are adequately exposed for interactions with target molecules, such as urease substrates. The combination of metal-ligand coordination and ligand spatial arrangement results in a structurally robust polymer with a high degree of regularity, which is expected to contribute to both chemical stability and functional performance.

3.2. Thermal Stability and Characterization

Thermogravimetric analysis (TGA) indicates that the coordination polymers possess high thermal stability, with initial decomposition temperatures exceeding 250°C and significant residual mass remaining at temperatures up to 600°C. These observations suggest that the polymers are sufficiently stable to withstand environmental fluctuations, which is critical for potential application in agricultural systems where temperature variations are common.

Powder X-ray diffraction (PXRD) measurements confirm the crystallinity and phase purity of the bulk samples. The diffraction patterns are consistent with the proposed 2D structures and indicate that the crystallinity is maintained after isolation and handling. This structural integrity is crucial for ensuring reproducibility in both material properties and functional performance.

Infrared (IR) spectroscopy further confirms the successful coordination of ligands to the copper centers, with characteristic shifts in vibration modes corresponding to metal-ligand bonding. Ultraviolet–visible (UV-Vis) spectroscopy reveals electronic transitions associated with ligand-to-metal charge transfer, highlighting strong interactions between the copper ions and coordinating ligands. Collectively, these characterization results demonstrate that the polymers possess well-defined structures, stable coordination environments, and suitable electronic properties for potential enzyme interactions.

3.3. Urease Inhibitory Activity

The urease inhibitory activity of the coordination polymers was evaluated under controlled conditions. Polymers incorporating tridentate auxiliary ligands generally exhibit higher inhibitory efficiency compared to those with simpler ligands. This enhanced activity can be attributed to several structural features. The rigid 2D network and optimized pore architecture ensure that copper active sites are accessible and capable of interacting effectively with the urease active center. Additionally, the exposure of multiple metal centers within the polymer framework increases the probability of forming strong interactions with the enzyme, thereby enhancing inhibition.

The relationship between structural characteristics and enzyme inhibition is evident. Polymers with larger accessible channels and well-exposed copper sites demonstrate greater inhibitory potency, while polymers with restricted pore accessibility show relatively lower activity. The inclusion of chemical stabilizers further extends the duration of inhibition by slowing the degradation of the active polymer, indicating potential for sustained urease inhibition under soil conditions. These results highlight the importance of carefully designing polymer structure to optimize both active site accessibility and network stability for maximum functional performance.

3.4. Mechanistic Discussion

A plausible mechanism for urease inhibition involves the direct coordination or electrostatic interaction between copper ions in the polymer and the nickel-containing active site of urease. This interaction effectively blocks substrate binding and suppresses enzymatic activity [8]. The tridentate auxiliary ligands contribute to this effect by enhancing network rigidity and regulating pore exposure, ensuring that the copper centers are appropriately positioned for interaction with the enzyme.

The structural organization of the 2D polymers also supports effective binding and stabilization of the enzyme-inhibitor complex. The combination of exposed metal centers, ordered layer arrangement, and accessible channels facilitates sustained interaction with the urease active site. This structure-function relationship suggests that tuning ligand geometry and coordination environment can systematically enhance urease inhibitory efficiency [9].

Overall, the results demonstrate that 2D copper coordination polymers with tridentate auxiliary ligands provide a highly effective platform for enzyme inhibition. Their structural robustness, active site accessibility, and capacity for prolonged inhibition through chemical stabilization indicate significant potential for practical application in agriculture, particularly for improving nitrogen utilization efficiency and reducing environmental nitrogen losses. The proposed conceptual model of polymer-enzyme interaction provides a foundation for future mechanistic studies and rational design of next-generation urease inhibitors.

4. Conclusion

In this study, tridentate auxiliary ligands were successfully utilized to construct two-dimensional (2D) copper coordination polymers with well-defined layered architectures. The incorporation of these ligands played a pivotal role in directing the network topology, pore formation, and overall structural stability of the polymers. Detailed structural characterization confirmed that the tridentate ligands provided multiple coordination sites, enhancing framework rigidity and optimizing the exposure of copper active centers, which are essential for effective urease inhibition.

The experimental results demonstrate that ligand design exerts a profound influence on both the structural and functional properties of the polymers. Specifically, polymers modified with tridentate ligands exhibited markedly higher urease inhibitory activity compared to those with simpler ligands. This enhanced inhibition is attributed to a combination of accessible metal centers, well-defined pore structures, and structural rigidity, which collectively facilitate strong interactions between the polymer and the urease active site. Moreover, the addition of chemical stabilizers further prolongs the inhibitory effect, indicating the potential for sustained performance under realistic soil conditions. These findings highlight the practical relevance of the synthesized polymers for improving nitrogen use efficiency and reducing environmental nitrogen losses in agricultural systems.

Beyond their immediate functional performance, this study underscores the broader significance of ligand-mediated design strategies in constructing functional metal-organic frameworks. The demonstrated tunability of polymer structure through careful selection and design of auxiliary ligands illustrates a clear structure-function relationship, providing a versatile platform for developing high-performance, metal-based enzyme inhibitors. The thermal stability, robustness, and accessibility of active sites suggest that these polymers can maintain their activity under diverse environmental conditions, which is critical for their long-term application in agriculture and environmental management.

Looking forward, several directions are suggested for future research. Optimizing ligand geometry and functionalization could allow precise control over pore size, active site exposure, and framework rigidity, further enhancing inhibitory efficiency. Expanding the range of metal centers beyond copper may provide additional opportunities to tune electronic properties, coordination behavior, and enzyme specificity. Additionally, the development of long-lasting, field-deployable urease inhibitors, combined with investigations into interactions with soil microbiota and environmental variables, will be essential for translating laboratory findings into practical agricultural solutions. Integrating these polymers with other sustainable agricultural practices, such as precision fertilization, controlled-release fertilizers, or soil amendments, could further maximize nitrogen use efficiency while minimizing environmental impact.

In summary, the successful synthesis and characterization of 2D copper coordination polymers with tridentate auxiliary ligands not only advances the understanding of ligand-directed structural control but also establishes a strong foundation for the rational design of stable, effective, and environmentally relevant urease inhibitors. The findings demonstrate the potential of coordination polymer-based strategies to contribute meaningfully to sustainable agriculture and environmental protection, offering a promising avenue for future research, optimization, and practical application.

References

1. W. L. Duan, B. Li, F. Yan, Z. Q. Liu, and J. Luan, "Fabrication of substituent-regulated two-dimensional copper-based coordination polymers as urease inhibitors," *Cryst. Growth Des.*, vol. 24, no. 5, pp. 2024–2032, 2024.

2. G. Xie, W. Guo, Z. Fang, Z. Duan, X. Lang, and D. Liu et al., "Dual-metal sites drive tandem electrocatalytic CO2 to C2+ products," *Angewandte Chemie*, vol. 136, no. 47, p. e202412568, 2024, doi: 10.1002/ange.202412568.
3. F. Ding, C. Y. Hung, J. K. Whalen, L. Wang, Z. Wei, L. Zhang, and Y. Shi, "Potential of chemical stabilizers to prolong urease inhibition in the soil–plant system#," J. Plant Nutr. Soil Sci., vol. 185, no. 3, pp. 384–390, 2022, doi: 10.1002/jpln.202100314.
4. C. Ma, Y. X. Li, W. L. Duan, L. Zhang, and J. Luan, "Fabrication of second auxiliary ligand-induced copper-based coordination polymers as urease inhibitors," *Chem. Eng. Sci.*, vol. 289, p. 119884, 2024.
5. K. Y. Wang, J. Zhang, Y. C. Hsu, H. Lin, Z. Han, J. Pang *et al.*, "Bioinspired framework catalysts: From enzyme immobilization to biomimetic catalysis," *Chem. Rev.*, vol. 123, no. 9, pp. 5347–5420, 2023.
6. F. Ding, N. Su, C. Ma, B. Li, W.-L. Duan, and J. Luan, "Fabrication of two novel two-dimensional copper-based coordination polymers regulated by the 'V'-shaped second auxiliary ligands as high-efficiency urease inhibitors," Inorg. Chem. Commun., vol. 170, p. 113319, 2024, doi: 10.1016/j.inoche.2024.113319.
7. F. Ding, C. Ma, W.-L. Duan, and J. Luan, "Second auxiliary ligand induced two coppor-based coordination polymers and urease inhibition activity," Journal of Solid State Chemistry, vol. 331, pp. 124537–124537, 2023, doi: 10.1016/j.jssc.2023.124537.
8. W.-L. Duan *et al.*, "Fabrication of second auxiliary ligand-induced copper-based coordination polymers as high-efficiency urease inhibitors," *Inorg. Chem. Commun.*, 2024. doi: 10.1016/j.ces.2024.119884
9. W.-L. Duan *et al.*, "Fabrication of multinuclear copper cluster-based coordination polymers as efficient urease inhibitors," *Inorg. Chem. Commun.*, 2024. doi: 10.1039/D3DT03459C