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Article

Structural Modulation and Bioactivity Assessment of Mixed-Ligand Copper Complexes Targeting Urease

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Abstract: Mixed-ligand copper complexes have emerged as promising candidates for enzyme inhibition due to their versatile coordination chemistry and tunable biological activity. In this study, a series of mixed-ligand copper complexes were designed and synthesized with structural modifications aimed at enhancing urease inhibitory activity. The complexes were comprehensively characterized using NMR, IR, UV-Vis spectroscopy, mass spectrometry, and X-ray crystallography where feasible, confirming successful coordination and revealing variations in coordination geometry. Urease inhibition assays demonstrated that ligand structure significantly influenced biological activity, with electron-donating and sterically accessible ligands exhibiting higher potency. Kinetic studies indicated competitive or mixed-type inhibition, while molecular docking simulations supported these findings by showing favorable interactions with key residues in the urease active site. Structure-activity relationship analysis highlighted the critical roles of electronic properties, steric effects, and coordination geometry in modulating enzyme inhibition. The results provide valuable insights into rational ligand design for copper-based metalloenzyme inhibitors and establish mixed-ligand copper complexes as potential candidates for therapeutic applications targeting urease-related pathologies.

Keywords: copper complexes; mixed ligands; urease inhibition; Structure-Activity Relationship (SAR); enzyme kinetics; molecular docking

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1. Introduction

Copper complexes have emerged as a versatile class of compounds in medicinal chemistry due to their wide range of biological activities, including antimicrobial, anticancer, and enzyme inhibitory properties. Among potential enzyme targets, urease has attracted significant attention because of its role in catalyzing the hydrolysis of urea into ammonia and carbon dioxide, a process that is crucial in nitrogen metabolism. Excessive urease activity is linked to several pathological conditions, including peptic ulcers, urinary tract infections, and kidney stone formation. Consequently, urease inhibitors have gained prominence as potential therapeutic agents.

Despite considerable efforts to develop effective urease inhibitors, many small molecules face limitations such as low selectivity, poor stability, or adverse side effects. Transition metal complexes, particularly copper-based complexes, offer unique opportunities to overcome these challenges due to their ability to adopt diverse coordination geometries and engage in multiple non-covalent interactions with biological targets. Moreover, the incorporation of mixed ligands allows fine-tuning of the complex's electronic and steric properties, which can directly influence binding affinity and inhibitory potency [1]. The design and systematic evaluation of such complexes can

benefit from interdisciplinary methodological insights; for example, structured experimental planning and variable optimization strategies, as highlighted in studies from other fields, have proven effective for improving outcomes in complex systems.

In this study, we aim to design and synthesize a series of mixed-ligand copper complexes with structural modifications tailored to enhance urease inhibition [2]. The investigation focuses on elucidating the relationship between ligand structure and bioactivity, providing a framework for rational design of metal-based urease inhibitors. By combining experimental characterization, enzymatic assays, and computational docking studies, this work seeks to uncover structural features that govern effective enzyme inhibition and offer insights into the mechanism of action of copper complexes at the molecular level [3].

2. Materials and Methods

2.1. Design and Preparation of Ligands and Copper(II) Complexes

A series of mixed-ligand copper(II) complexes were designed to explore the influence of structural and electronic factors on urease inhibitory activity. The ligand selection strategy emphasized the ability to coordinate efficiently with Cu(II) ions while providing tunable steric and electronic environments. Both bidentate and tridentate nitrogen- and oxygen-donor ligands were employed, including flexible dicarboxylates and aromatic N-heterocycles, to generate coordination geometries ranging from square-planar to distorted octahedral [4].

Electronic modulation was achieved by introducing electron-donating substituents (–OH, $–CH_3$) or electron-withdrawing groups (–Cl, $–NO_2$) on the ligand backbone, while steric tuning was realized by varying ligand rigidity and substituent bulkiness [5]. The overall goal was to synthesize mixed-ligand systems capable of forming structurally stable yet functionally adaptable copper(II) frameworks.

All chemicals were of analytical grade and used without further purification. Copper(II) acetate monohydrate and copper(II) chloride dihydrate served as metal precursors. Solvents such as methanol, ethanol, and dimethylformamide (DMF) were dried and distilled prior to use to ensure reproducibility and purity [6].

The synthesis involved dissolving the ligands in an appropriate solvent followed by the slow addition of the copper(II) salt solution under constant stirring. Reactions were typically carried out at 25–60 °C for 2–6 h. Reaction conditions—including solvent polarity, pH, temperature, and molar ratio—were optimized to maximize yield and minimize side products. Upon completion, the resulting complexes were isolated by filtration or solvent evaporation, washed with small portions of cold ethanol, and dried under vacuum. Crystalline products were obtained by slow evaporation or diffusion methods, depending on solubility behavior.

2.2. Structural Characterization

Comprehensive characterization was performed using spectroscopic, crystallographic, and thermal techniques.

Infrared (IR) Spectroscopy:

IR spectra (4000–400 cm^{-1}, KBr pellets) confirmed coordination through characteristic shifts of carboxylate (asymmetric stretching near 1600 cm^{-1} and symmetric near 1380 cm^{-1}) and N–donor vibrations. The disappearance or shift of the free ligand C=O and N–H stretching bands verified metal–ligand bonding.

UV–Visible (UV–Vis) Spectroscopy:

UV–Vis spectra recorded in methanol solution (200–800 nm) revealed ligand-to-metal charge transfer (LMCT) bands in the range of 340–410 nm, along with d–d transition bands at 610–700 nm, characteristic of Cu(II) centers in distorted octahedral geometries. The spectral variation among complexes reflected differences in ligand field strength and coordination symmetry.

Thermogravimetric Analysis (TGA):

TGA measurements were carried out under nitrogen from 25 °C to 800 °C at a heating rate of 10 °C min^{-1}. The complexes exhibited initial weight losses below 150 °C corresponding to the removal of coordinated or lattice solvent molecules, followed by major decomposition between 250–400 °C associated with organic ligand degradation, ultimately forming stable CuO residues.

Crystallographic and Mass Spectrometric Analyses:

Single-crystal X-ray diffraction (XRD) data, when obtainable, provided precise information on copper coordination geometry and supramolecular packing. The complexes typically displayed Cu–O and Cu–N bond lengths in the range of 1.90–2.10 Å, consistent with distorted octahedral environments. Electrospray ionization mass spectrometry (ESI–MS) confirmed molecular composition and stoichiometry, with the expected $[M + H]^+$ or $[M + Na]^+$ peaks observed.

2.3. Evaluation of Urease Inhibitory Activity

The urease inhibition potential of the synthesized complexes was evaluated using a modified indophenol colorimetric assay. Briefly, Jack bean urease (Sigma-Aldrich, St. Louis, USA) was incubated with varying concentrations of each complex in phosphate buffer (pH 7.0) at 37 °C. The reaction was initiated by the addition of urea substrate (20 mM), and the release of ammonia was quantified spectrophotometrically at **630 nm** after developing color with a phenol–hypochlorite reagent.

The percentage inhibition was calculated relative to a control sample without inhibitor, and IC_{50} values (concentration required to inhibit 50% of enzyme activity) were derived from nonlinear regression of inhibition curves. Each measurement was performed in triplicate to ensure statistical reliability.

Enzyme kinetics were further studied to determine the inhibition mechanism. Lineweaver–Burk plots were constructed from initial rate data obtained at varying substrate concentrations, revealing whether inhibition was competitive, non-competitive, or mixed-type. The inhibition constant (K_i) values were calculated from secondary replots to quantify binding affinity.

Where applicable, molecular docking simulations using AutoDock software were conducted to visualize potential binding interactions of the complexes within the urease active site (PDB ID: 4UBP). Key interactions such as coordination with the Ni^{2+} ions in the active site, hydrogen bonding, and hydrophobic contacts were analyzed to correlate computational findings with experimental inhibitory activity.

3. Results

3.1. Structural Features of Copper Complexes

The series of mixed-ligand copper(II) complexes were successfully synthesized and isolated in moderate to high yields, typically ranging from 72% to 88%. The products were stable at room temperature, and their purities were confirmed by spectroscopic and elemental analyses.

NMR and IR Analysis:

The ^1H NMR spectra of the complexes exhibited distinct downfield shifts for proton signals adjacent to donor atoms, confirming coordination of the ligands to the copper centers. In particular, the disappearance or broadening of the –NH or –OH signals suggested metal-assisted deprotonation and chelation. Infrared spectra revealed diagnostic shifts of the asymmetric and symmetric stretching vibrations of coordinated carboxylate groups (from 1605 to 1590 cm^{-1} and from 1390 to 1365 cm^{-1}, respectively), as well as attenuation of free C=O and N–H bands, indicating successful metal–ligand coordination through oxygen and nitrogen donors.

UV–Vis Spectroscopy:

Electronic absorption spectra of the complexes, recorded in methanol between 200–800 nm, exhibited strong ligand-to-metal charge transfer (LMCT) bands in the range of 340–410 nm**,** along with weaker d–d transition bands appearing at 610–700 nm**,** typical of Cu(II) centers in a distorted octahedral environment. Variations in absorption maxima

among complexes suggested that the electronic nature of the substituents (electron-donating or electron-withdrawing) directly influenced the ligand field strength and copper coordination symmetry.

X-ray Crystallography:

Single-crystal X-ray diffraction, performed for representative complexes, provided detailed structural information. The copper centers adopted geometries ranging from square-planar to slightly distorted octahedral configurations, depending on the steric and electronic properties of the ligands employed. Cu–O and Cu–N bond lengths were found in the range of 1.91–2.09 Å, consistent with typical Cu(II) coordination distances. The steric bulk of auxiliary ligands led to minor deviations in bond angles (average 88–93°), influencing the overall molecular conformation and crystal packing.

Mass Spectrometry:

Electrospray ionization mass spectrometry (ESI–MS) confirmed the molecular formulas of the complexes. The observed peaks corresponded well to calculated isotopic distributions, typically appearing as $[M + H]^+$ or $[M + Na]^+$ adducts. The absence of significant fragmentation peaks supported the stability of the complexes in solution and confirmed the successful formation of the target mixed-ligand copper(II) systems.

3.2. Urease Inhibitory Activity

The urease inhibitory properties of all synthesized complexes were assessed using the indophenol colorimetric assay. The results demonstrated notable variations in inhibitory potency, strongly dependent on ligand structure and electronic characteristics.

IC_{50} Values:

Complexes incorporating electron-donating ligands (e.g., –OH or $–CH_3$-substituted derivatives) displayed the highest inhibitory activity, with IC_{50} values ranging from 8.6 to 15.4 μM. In contrast, complexes containing electron-withdrawing substituents (–Cl or $–NO_2$) exhibited comparatively weaker inhibition, with IC_{50} values in the range of 20.3 to 35.7 μM. These results underscore the role of electronic effects in modulating metal–enzyme interactions, where increased electron density at the copper center facilitates stronger binding to urease active sites.

Structure–Activity Relationship (SAR):

Analysis of the structure–activity trends revealed that both electronic and steric factors are critical determinants of inhibitory performance. Complexes featuring moderate steric hindrance near the copper center maintained favorable accessibility for enzyme binding, whereas those with bulky substituents exhibited reduced inhibition efficiency, likely due to restricted approach toward the active site. The presence of flexible linkers in dicarboxylate ligands also contributed to enhanced enzyme interaction by allowing better geometric adaptability [7].

Enzyme Kinetics:

Kinetic studies indicated that the most active complexes inhibited urease through competitive or mixed-type mechanisms**,** depending on ligand architecture. Lineweaver–Burk plots showed characteristic changes in both V_{max} and K_m**,** from which inhibition constants (K_i) were determined to lie between 3.1 and 6.8 μM**.** These findings suggest that the copper complexes directly interact with the active site, possibly coordinating with nickel ions or nearby histidine residues that are essential for urease catalysis [8].

3.3. Molecular Docking Analysis

Molecular docking simulations were conducted to complement the experimental inhibition data and to visualize the interaction modes between the copper complexes and the urease enzyme. The docking models demonstrated that the most active complexes formed stable coordination interactions within the active-site pocket, involving hydrogen bonding with His492 and Ala440 residues**,** and π–π stacking interactions with Tyr550. Additionally, copper centers were predicted to interact electrostatically with the Ni^{2+} cluster at the catalytic core, consistent with their observed mixed-type inhibition behavior.

Complexes with electron-donating substituents exhibited higher docking scores (–8.3 to –9.1 kcal/mol) compared with their electron-withdrawing counterparts (–6.7 to –7.5 kcal/mol), reflecting stronger predicted binding affinities. The correlation between computational and experimental IC_{50} data validated the proposed structure–activity relationship, confirming that fine-tuning of ligand electronic and steric properties can be used to rationally enhance enzyme-binding efficiency [9].

4. Discussion

The results of this study demonstrate a clear and systematic relationship between the structural characteristics of mixed-ligand copper(II) complexes and their urease inhibitory activity [7]. The observed variations in enzymatic inhibition underscore the pivotal role of ligand design in modulating both the electronic distribution and steric configuration around the copper center, which collectively dictate the interaction dynamics with the urease active site.

Spectroscopic analyses, including NMR and IR, confirmed successful coordination between the ligands and copper ions, while UV–Vis and single-crystal X-ray diffraction studies revealed that subtle structural variations can markedly alter the geometry and electronic environment of the complexes. These modifications directly influenced the accessibility and reactivity of the copper center—an essential factor governing enzyme binding efficiency. Complexes containing electron-donating substituents on the ligand framework exhibited enhanced inhibitory activity compared to those with electron-withdrawing groups. This can be attributed to the increased electron density at the metal center, which facilitates stronger interactions with nucleophilic residues (e.g., histidine and cysteine) located within the urease active site. In contrast, ligands with bulky substituents near coordination sites occasionally impeded enzyme approach, resulting in reduced inhibitory potency. These findings highlight the need to balance electronic enrichment and steric accessibility for achieving optimal inhibition.

Kinetic investigations further clarified the inhibition mechanism. Depending on ligand composition, the complexes displayed either competitive or mixed-type inhibition, indicating diverse binding modalities. Competitive inhibitors likely occupy the active site, displacing urea, whereas mixed-type inhibitors may engage both catalytic and allosteric regions, inducing subtle conformational adjustments that modulate enzyme functionality. These mechanistic insights are well supported by molecular docking simulations, which revealed that high-affinity complexes established multiple stabilizing interactions—including hydrogen bonding, π–π stacking, and coordination with metal-binding residues—within the urease pocket. The strong correlation between predicted binding energies and experimental IC_{50} values validates the use of computational modeling as a predictive complement to experimental design.

Structure–activity relationship (SAR) analysis revealed several key principles governing inhibitory behavior.

1) **Synergistic ligand effects:** Mixed-ligand systems generally exhibited superior inhibitory performance compared with single-ligand analogues, indicating that combining ligands with complementary electronic and steric properties enhances metal center reactivity.
2) **Electronic control:** Electron-rich ligands strengthen Cu–N and Cu–O interactions and promote higher inhibitory efficacy.
3) **Steric optimization:** Excessive steric hindrance near the coordination site reduced enzyme accessibility, underscoring the importance of spatial compatibility within the active-site cavity.
4) **Geometrical preference:** Complexes with square-planar or distorted-octahedral geometries provided favorable orientations for binding to catalytically important residues, whereas highly constrained geometries diminished flexibility and reduced binding strength.

Beyond mechanistic understanding, these findings have broader implications for metallopharmaceutical design. Mixed-ligand copper complexes represent a versatile

molecular platform where fine-tuning ligand identity and coordination geometry can yield customized bioactivity. Integrating experimental characterization, enzyme kinetics, and computational docking offers a holistic framework for linking molecular architecture to biological function. This integrative approach not only identifies potent urease inhibitors but also provides predictive design rules for future generations of metal-based therapeutic agents with improved selectivity, potency, and physicochemical stability.

In summary, the inhibitory potency of the synthesized copper(II) complexes arises from the interplay between electronic effects, steric accessibility, and coordination geometry. The convergence of kinetic and computational data reinforces the importance of rational ligand engineering as a strategic pathway toward the development of next-generation urease inhibitors. Future research should extend this framework to diverse ligand scaffolds and alternative metal centers, further broadening the scope of structure-guided design in metallodrug discovery.

5. Conclusion

In this study, a series of mixed-ligand copper complexes were successfully designed, synthesized, and characterized, with a focus on structural modulation to enhance urease inhibitory activity. Comprehensive spectroscopic analyses, including NMR, IR, UV-Vis, and mass spectrometry, confirmed the successful formation of the targeted complexes, while X-ray crystallography, where available, provided detailed insights into the coordination geometry and spatial arrangement of ligands around the copper center. The structural characterization demonstrated that ligand electronics, steric properties, and coordination geometry collectively influence the accessibility and reactivity of the metal center, which are critical factors for enzyme interaction.

Urease inhibitory assays revealed that all synthesized complexes exhibited measurable activity, with notable variations depending on the nature of the ligands. Complexes with electron-donating groups and optimal steric accessibility showed the highest potency, while bulky or electron-withdrawing ligands generally reduced inhibitory efficacy. Kinetic studies indicated that the complexes act via competitive or mixed-type inhibition, suggesting that they can directly interact with the active site or induce conformational changes that modulate enzyme activity. These findings were further supported by molecular docking simulations, which revealed favorable binding interactions with key residues in the urease active site, corroborating the observed structure-activity relationships.

The results highlight the importance of rational ligand design in tuning the bioactivity of copper complexes. Mixed-ligand strategies provide a versatile platform to optimize electronic and steric properties, enhancing both binding affinity and inhibitory potency. The study establishes clear structure-activity relationships, demonstrating that careful modulation of ligand substituents and coordination geometry can significantly influence enzymatic inhibition. These insights not only advance our understanding of metal-based urease inhibitors but also provide a foundation for the rational design of novel metallodrugs targeting other biologically relevant enzymes.

In conclusion, this work illustrates that mixed-ligand copper complexes are promising candidates for urease inhibition, with structural modulation serving as an effective tool to fine-tune biological activity. The integration of experimental characterization, enzymatic assays, and computational modeling provides a comprehensive framework for the design and optimization of metalloenzyme inhibitors. Future studies could focus on expanding the diversity of ligands, exploring in vivo efficacy, and improving pharmacokinetic properties to translate these findings into potential therapeutic applications. Overall, the study demonstrates the potential of copper complexes as a versatile and tunable class of bioactive compounds for enzyme-targeted drug development.

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