SYNTHESIS AND INVESTIGATION OF MIXED-LIGAND COMPLEX COMPOUNDS INVOLVING FLAVONOIDS AND SELECTED AMINO ACIDS

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Abstract. *Mixed-ligand coordination compounds based on biologically active natural ligands have attracted significant attention in modern bioinorganic and coordination chemistry, owing to their enhanced physicochemical and pharmacological properties relative to mono-ligand analogs. This study aims to synthesize novel mixed-ligand complexes of Cu(II), Ni(II), Co(II), and Zn(II) ions with flavonoids (kaempferol and quercetin) and amino acids (glycine and β-alanine), and to characterize them by multiple physicochemical methods. Complexes were synthesized in a water-ethanol medium at pH 6.5–7.5, 40–60 °C, for 2–4 h at a 1:1:1 (M:L1:L2) molar ratio. Characterization employed XRF spectrometry, UV-Vis spectrophotometry, FTIR spectroscopy, DFT calculations, and molecular docking. Ten mixed-ligand complexes were successfully synthesized. XRF confirmed the presence of the metal centers; UV-Vis spectra displayed bathochromic shifts and new d–d transition bands; FTIR data evidenced coordination via carboxylate, carbonyl, and phenolate groups. DFT calculations yielded HOMO–LUMO gaps of ~3.73–3.74 eV for both flavonoids, and molecular docking demonstrated strong protein-binding affinities. The synthesized complexes show promising potential for pharmaceutical and biomedical applications.*

Keywords: *mixed-ligand complexes; flavonoids; kaempferol; quercetin; amino acids; glycine; β-alanine; Cu(II); Ni(II); Co(II); Zn(II); DFT; molecular docking; IR spectroscopy; XRF*

1. Introduction

The design and study of mixed-ligand coordination compounds incorporating biologically active molecules constitute one of the most dynamic research areas in contemporary bioinorganic chemistry. The simultaneous coordination of two structurally distinct ligands to a single metal center frequently yields compounds with synergistically enhanced biological, spectroscopic, and structural properties that

exceed those of their mono-ligand counterparts [1,2]. This “mixed-ligand strategy” has been widely exploited to improve solubility, bioavailability, and cellular uptake of potential metallodrugs.

Flavonoids are a broad family of naturally occurring polyphenolic compounds characterized by the benzo-γ-pyrone (chromon) C6–C3–C6 phenylpropanoid framework. Their wide distribution in the plant kingdom, combined with well-documented antioxidant, anti-inflammatory, anticancer, and cardioprotective activities, has attracted extensive scientific attention [3,4]. Among flavonoids, kaempferol (3,5,7,4'-tetrahydroxyflavone, $C_{15}H_{10}O_6$) and quercetin (3,5,7,3',4'-pentahydroxyflavone, $C_{15}H_{10}O_7$) are particularly prominent representatives of the flavonol subclass. Both compounds exhibit potent free-radical scavenging capacity, inhibit pro-inflammatory cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, and have demonstrated antitumor activity through modulation of apoptotic pathways and cell-cycle arrest [5,6].

Amino acids represent a complementary and biologically relevant class of bifunctional ligands that readily form stable chelate systems with transition metal ions through their carboxylate ($–COO^–$) and amine ($–NH_2$) donor groups. Glycine, the simplest α-amino acid, and its β-isomer β-alanine are well-studied ligands known to form robust five- or six-membered chelate rings upon coordination to d-block metal centers, significantly enhancing the stability, solubility, and biological compatibility of the resulting complexes [7,8].

The integration of flavonoids and amino acids as co-ligands within a single metal coordination sphere offers a rational synthetic strategy for accessing new coordination compounds with tunable electronic, structural, and pharmacological properties. Transition metal ions such as Cu(II), Ni(II), Co(II), and Zn(II) are of particular interest due to their biological relevance, variable coordination geometries, and well-characterized spectroscopic signatures that facilitate structural analysis [9].

Despite growing interest in flavonoid–metal complexes and amino acid–metal complexes individually, the coordination behavior, spectral characterization, and quantum-chemical properties of ternary mixed-ligand systems involving both classes of ligands with d-block metal ions remain incompletely explored. The present work addresses this gap by reporting the synthesis, characterization, and computational analysis of ten novel mixed-ligand complexes based on Co(II), Ni(II), Cu(II), and Zn(II) with kaempferol or quercetin as one ligand and glycine or β-alanine as the second ligand.

2. Materials and Methods

2.1. Reagents and Instrumentation

All chemicals were of analytical reagent grade and used as received. Metal salts $CoCl_2 \cdot 6H_2O$ (MW = 237.93), $NiCl_2 \cdot 6H_2O$ (MW = 237.69), $CuCl_2 \cdot 2H_2O$ (MW = 170.48), and $ZnCl_2$ (MW = 136.29) were of analytical purity (≥98%). Kaempferol (MW = 286.24, ≥98%) and quercetin (MW = 302.24, ≥98%), glycine (MW = 75.03), and β-alanine (MW = 89.09) were obtained from commercial suppliers. Distilled water and 96% ethanol served as solvents. pH was monitored throughout using a calibrated digital pH meter.

Infrared spectra were recorded on a Shimadzu IRaffinity-1S Fourier-transform infrared spectrometer in the 400–4000 cm^{-1} range using KBr pellets. UV-Vis spectra were measured on a Shimadzu UV-2600i spectrophotometer (300–800 nm). Elemental composition was determined by energy-dispersive X-ray fluorescence (XRF) using a Rigaku NEX DE spectrometer. Fluorescence measurements were made on a Shimadzu RF-6000 spectrofluorimeter. All instruments were calibrated prior to measurement.

2.2. Synthesis of Mixed-Ligand Complexes

A general procedure was employed for all syntheses. A 0.1 M aqueous solution of the metal chloride salt was prepared and equilibrated at 40–60 °C with constant magnetic stirring. The quantities used were: $CoCl_2 \cdot 6H_2O$ and $NiCl_2 \cdot 6H_2O$ (0.238 g in 10 mL water); $CuCl_2 \cdot 2H_2O$ (0.170 g in 10 mL); $ZnCl_2$ (0.136 g in 10 mL). An aqueous solution of the amino acid (glycine: 0.075 g; β-alanine: 0.089 g; each in 10 mL water) was added dropwise to the metal solution. Subsequently, an ethanolic solution of the flavonoid (kaempferol: 0.086 g; quercetin: 0.090 g; each in 10 mL of 96% ethanol) was added dropwise with continuous stirring.

The pH was adjusted and maintained between 6.5 and 7.5 by dropwise addition of dilute NaOH or HCl solutions. This pH range was selected because it promotes partial deprotonation of the flavonoid phenolic groups, enabling effective metal coordination, while avoiding hydrolysis of the metal ion. The reaction mixture was stirred continuously for 2–4 hours. The resulting precipitates were collected by vacuum filtration, washed with distilled water and ethanol to remove unreacted starting materials, and dried at room temperature. The final metal-to-amino acid-to-flavonoid molar ratio was 1:1:1 for all ten complexes.

2.3. Computational Methods

Quantum chemical calculations were conducted within the framework of Density Functional Theory (DFT) at the B3LYP/6-311G(d,p) level of theory for both free flavonoid ligands. Full geometry optimization was performed in the gas phase. Frontier molecular orbital energies (E_HOMO, E_LUMO) and the energy gap (ΔE), as well as molecular electrostatic potential (MEP) maps, were calculated to identify electron-donor centers and coordination sites. Biological activity predictions were

made using the PASS Online server. Molecular docking was carried out using AutoDock Vina, and a 100 ns molecular dynamics simulation was performed for the kaempferol–protein complex to assess binding stability.

3. Results and Discussion

3.1. Synthesis and Physical Appearance

Ten mixed-ligand complexes were successfully obtained as stable colored precipitates. The observed colors — ranging from dark green-blue (Co/Ni/Cu complexes) to off-white/yellow (Zn complexes) and pink-violet (Co with β-alanine/kaempferol) — reflect the d-electron configuration of the respective metal centers and are consistent with the formation of new coordination environments. All precipitates were stable at ambient conditions.

Table 1. Synthesized mixed-ligand complexes and their physical properties

No.	Metal Salt	Amino Acid	Flavonoid	Color	pH	Reaction time
1	$CoCl_2 \cdot 6H_2O$	Glycine	Kaempferol	Dark green-blue	6.5–7.5	2–3 h
2	$NiCl_2 \cdot 6H_2O$	Glycine	Kaempferol	Green	6.5–7.5	2–4 h
3	$CuCl_2 \cdot 2H_2O$	Glycine	Kaempferol	Dark green-blue	6.5–7.5	2–4 h
4	$ZnCl_2$	Glycine	Kaempferol	Off-white/yellow	6.5–7.5	2–3 h
5	$NiCl_2 \cdot 6H_2O$	β-Alanine	Kaempferol	Green	6.5–7.5	2–4 h
6	$CoCl_2 \cdot 6H_2O$	β-Alanine	Kaempferol	Pink-violet	6.5–7.5	2–4 h
7	$CuCl_2 \cdot 2H_2O$	β-Alanine	Kaempferol	Blue-green	6.5–7.5	2–4 h
8	$ZnCl_2$	β-Alanine	Kaempferol	Off-white/yellow	6.5–7.5	2–3 h
9	$CoCl_2 \cdot 6H_2O$	Glycine	Quercetin	Dark pink-violet	6.5–7.5	2–4 h
10	$NiCl_2 \cdot 6H_2O$	Glycine	Quercetin	Green	6.5–7.5	2–4 h

3.2. XRF Elemental Analysis

X-ray fluorescence spectroscopy confirmed the presence of the respective metal centers in the synthesized complexes. For the Co(II)–glycine–quercetin complex, Co and Cl were quantified at 55.1% and 44.9% by mass, respectively. The higher cobalt content relative to chloride indicates that the organic ligands have partially displaced

chloride anions from the inner coordination sphere, with the remaining chloride serving as outer-sphere counter ions. For the Co(II)–glycine–kaempferol complex, Co and Cl mass fractions were 43.4% and 56.6%, suggesting a greater degree of chloride retention in this case, likely as both inner- and outer-sphere species.

Organic ligand atoms (C, H, N, O) were not detected by XRF, as their characteristic X-ray fluorescence energies lie below the detection threshold of the spectrometer under ambient conditions. The absence of extraneous metal signals in all spectra confirms the high purity of the synthesized products and the absence of metal-containing impurities.

3.3. UV-Vis Spectroscopic Analysis

UV-Vis spectroscopy is highly informative for characterizing flavonoid–metal complexes because free flavonoids exhibit characteristic absorption bands attributable to their aromatic chromophore systems. Free quercetin typically shows a Band II absorption (benzoyl system, $\pi \rightarrow \pi^*$) near 250–270 nm and a Band I absorption (cinnamoyl system, $\pi \rightarrow \pi^*$) near 360–380 nm. In the Co(II)–glycine–quercetin complex, a strong absorption was observed at 320–350 nm, and a broad, relatively weak band appeared near 520–540 nm. The bathochromic shift of the main flavonoid band indicates electronic perturbation through the 3-OH/4-C=O chelation site. The absorption near 520–540 nm, absent in the free ligand spectra, is assigned to d–d electronic transitions of the Co(II) center in the ligand field created by the coordinating donors.

The Co(II)–glycine–kaempferol complex showed analogous spectral features: intense absorption at 300–350 nm and a broad d–d transition near 520–530 nm. A gradual decrease in absorbance from 350 to 450 nm is consistent with ligand-to-metal charge transfer (LMCT) contributions from the phenolate oxygen donors. These observations collectively confirm the formation of new coordination bonds and are consistent with literature reports for Co(II) complexes of structurally similar flavonoids.

3.4. FTIR Spectroscopic Analysis

FTIR spectroscopy provided direct evidence for the involvement of specific functional groups in metal coordination. In the Co(II)–glycine–quercetin complex, the broad ν(O–H) band of quercetin phenolic groups, normally at 3270–3400 cm^{-1}, shifted to 3257 cm^{-1} in the complex with increased breadth, indicating vodorod bonding enhancement and coordination of the phenolic oxygen to the metal. Glycine carboxylate stretching vibrations appeared at $\nu_{as}(COO^-)$ = 1593 cm^{-1} and $\nu_{s}(COO^-)$ = 1384 cm^{-1}, giving $\Delta\nu$ = 209 cm^{-1}. This value exceeding 200 cm^{-1} is consistent with monodentate or asymmetric carboxylate coordination. New bands at 662, 625, 590,

558, and 520 cm^{-1}, absent in the free ligand spectra, are assigned to Co–O metal-ligand stretching vibrations.

In the Co(II)–glycine–kaempferol complex, the ν(O–H) region shifted to 3282–3148 cm^{-1}. The free C=O carbonyl band of kaempferol (normally 1655–1665 cm^{-1}) disappeared and was replaced by carboxylate-type bands at 1563 and 1413 cm^{-1}, indicating that the 4-keto oxygen participates in coordination through deprotonation. The C–O stretching vibrations of the flavonoid skeleton remained at 1174, 1090, and 998 cm^{-1}, confirming structural integrity of the flavonoid moiety within the complex. New Co–O stretching bands at 560–530 cm^{-1} provide further direct confirmation of metal–oxygen bond formation.

3.5. DFT Calculations and Molecular Docking

DFT/B3LYP/6-311G(d,p) calculations provided quantum-chemical characterization of the free flavonoid ligands. For quercetin, the HOMO energy was E_HOMO = −5.730 eV and the HOMO–LUMO gap was ΔE = 3.744 eV; for kaempferol, E_HOMO = −5.991 eV, E_LUMO = −2.264 eV, and ΔE = 3.726 eV. These intermediate gap values indicate that both ligands are electronically reactive yet sufficiently stable for biological activity. The HOMO electron density is predominantly localized on the oxygen-bearing functional groups and the aromatic π-system, identifying the 3-OH, 5-OH, 4-C=O, and catechol hydroxyl groups as the primary electron-donor and metal-binding sites. MEP maps confirm the highest negative electrostatic potential at these oxygen centers.

PASS Online toxicity modeling predicted low general toxicity (10–20%) and very high protective/antioxidant activity (70–88%) for both flavonoids, supporting their safety for pharmacological applications. Molecular docking of quercetin against the target protein demonstrated strong binding with key residues Ser138, Thr137, Val33, Asp99, Lys104, and Glu101 at hydrogen bond distances of 1.88–2.85 Å. A 100 ns molecular dynamics simulation of the kaempferol–protein complex confirmed structural stability throughout the simulation, with the ligand retained in the binding pocket with only minor conformational adjustments.

4. Conclusions

This study reports the synthesis and characterization of ten novel mixed-ligand coordination complexes based on Cu(II), Ni(II), Co(II), and Zn(II) with kaempferol or quercetin as one co-ligand and glycine or β-alanine as the second co-ligand. The principal findings are:

1. Optimal synthesis conditions were established: water-ethanol mixed solvent, pH 6.5–7.5, temperature 40–60 °C, 2–4 hours reaction time, 1:1:1 molar ratio. These

conditions promote partial deprotonation of the flavonoid phenolic groups to enable effective metal chelation.

2. XRF analysis confirmed the identity and purity of metal centers in all complexes. Chloride distribution patterns suggest partial inner-sphere displacement by the organic ligands, with residual chloride acting as outer-sphere counter ions.
3. UV-Vis spectroscopy confirmed complex formation through bathochromic shifts of flavonoid absorption bands and the appearance of new d–d transition bands in the 520–540 nm region, absent in the free ligand spectra.
4. FTIR analysis identified the amino acid carboxylate groups ($\Delta\nu > 200$ cm^{-1}), flavonoid carbonyl and phenolate oxygens, and new metal–oxygen stretching vibrations (500–700 cm^{-1}) as the functional groups participating in coordination.
5. DFT calculations established $\Delta E \approx 3.73–3.74$ eV and localized HOMO density on oxygen donor centers for both flavonoids, providing a quantum-chemical basis for their observed coordination behavior and antioxidant properties.
6. Molecular docking and dynamics simulations confirmed strong, stable protein-binding capability, supporting the pharmaceutical potential of these mixed-ligand frameworks.

Collectively, these results demonstrate that the mixed-ligand strategy combining flavonoids and amino acids with biologically relevant metal ions provides a versatile approach to new coordination compounds with potential applications in pharmacology, antioxidant therapy, and medicinal chemistry. Further biological evaluation in vitro and in vivo is warranted.

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