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SYNTHESIS, CHARACTERIZATION, BSA BINDING ACTIVITY AND ADME PREDICTIONS OF A NEW Co(II) COMPLEX WITH O-VANILLIN-BASED SCHIFF BASE LIGAND

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Abstract

This study reports the synthesis, structural characterization, bovine serum albumin (BSA) binding activity, and pharmacokinetic properties of an o-vanillin-based Schiff base ligand and its Co(II) complex. The ligand (H_2L) was synthesized via condensation reaction between isonitrosophenyl hydrazine and o-vanillin, and the Co(II) complex [$Co(HL)_2$] was prepared by reacting the ligand with cobalt(II) acetate monohydrate. The ligand was characterized by elemental analysis, UV-Vis, FTIR, NMR spectroscopy, while Co(II) complex was characterized by elemental analysis, UV-Vis, magnetic susceptibility, FTIR and mass spectrometry, confirming successful complex formation with octahedral geometry. BSA binding studies of Co(II) complex demonstrated moderate binding (K_{bin}=8.74x10^3 M^{-1}) via static quenching mechanism, supported by thermodynamic calculations (ΔG=-22.49 kJ.mol^{-1}). The ligand also exhibited BSA binding with a lower affinity (K_{bin}=1.36x10^3 M^{-1}), indicating that metal coordination significantly enhances protein interaction. In silico ADME predictions of Co(II) complex indicated promising pharmacokinetics characterized by a molecular weight of 651.53 g/mol, polar surface area (TPSA = 151.54 Å^2), moderate flexibility, and reasonable physicochemical stability. However, limited gastrointestinal absorption and lack of blood-brain barrier permeability, suggest potential suitability for peripheral therapeutic applications. Future studies will involve detailed cytotoxicity assays and pharmacokinetic evaluations to validate the therapeutic efficacy and safety profile of this promising Co(II) complex and Schiff base ligand.

Keywords: Co(II) Complex, o-Vanillin, Schiff Base, BSA Binding, ADME

O-VANİLİN TEMELLİ SCHIFF BAZI LİGANDI İLE YENİ BİR Co(II) KOMPLEKSİNİN SENTEZİ, KARAKTERİZASYONU, BSA BAĞLANMA AKTİVİTESİ VE ADME TAHMİNLERİ

Özet

Bu çalışma, o-vanillin bazlı bir Schiff bazı ligandının ve Co(II) kompleksinin sentezi, karakterizasyonu, bovin serum albümini (BSA) ile etkileşimi ve farmakokinetik özelliklerini raporlamaktadır. Ligand (H_2L), izonitrozofenil hidrazin ile o-vanillin arasındaki kondenzasyon reaksiyonu ile sentezlenmiş; Co(II) kompleksi [$Co(HL)_2$] ise sentezlenen ligandın kobalt(II) asetat monohidrat ile tepkimesiyle hazırlanmıştır. Ligand; elementel analiz, UV-Vis, FTIR, NMR spektroskopisi ile, Co(II) kompleksi ise elementel analiz, UV-Vis, FTIR spektroskopisi, manyetik duyarlılık ölçümü ve kütle spektrometrisi ile karakterize edilmiş ve bu sayede kompleksin oktahedral geometriye sahip olduğu doğrulanmıştır. Co(II) kompleksinin BSA ile bağlanma çalışmaları, statik söndürme mekanizması aracılığıyla orta düzeyde bir bağlanmayı (K_{bin}= 8.74x10^3 M^{-1}) ortaya koymuş; bu durum termodinamik hesaplamalarla (ΔG=–22.49 kJ.mol^{-1}) desteklenmiştir. Ayrıca, ligand da daha düşük bir bağlanma afinitesiyle (K_{bin}=1.36x10^3 M^{-1}) BSA ile etkileşim göstermiştir; bu da metal koordinasyonunun protein etkileşimini önemli ölçüde artırdığını göstermektedir. Co(II) kompleksine ait ADME tahminleri, 651.53 g/mol moleküler ağırlık, 151.54 Å^2 polar yüzey alanı (TPSA), orta düzeyde esneklik ve iyi düzeyde fizikokimyasal kararlılık gibi umut verici farmakokinetik özellikleri göstermiştir. Bununla birlikte, sınırlı gastrointestinal emilim ve kan-beyin bariyeri geçirgenliğinin olmaması, bu kompleksin periferik tedavi uygulamaları için uygun olabileceğini düşündürmektedir. Gelecek çalışmalarda, bu umut verici Co(II) kompleksi ve Schiff bazı ligandının terapötik etkinliğini ve güvenlik profilini doğrulamak amacıyla ayrıntılı sitotoksisite testleri ve farmakokinetik değerlendirmeler yapılacaktır.

Anahtar Kelimeler: Co(II) Kompleksi, O-Vanilin, Schiff Bazı, BSA Bağlanma, ADME

Cite

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1. Introduction

Transition metal complexes have gained significant attention in medicinal chemistry due to their diverse biological activities and potential therapeutic applications [1-3]. Unlike conventional small-molecule drugs, transition metal-based therapeutics offer distinct advantages, including overcoming drug resistance, enhancing efficacy, and reducing toxicity [4, 5]. Among these, cobalt(II) complexes have emerged as particularly promising candidates due to their unique redox properties, coordination versatility, and bioactive potential, especially in anticancer and antimicrobial research [6-10]. In contrast to other transition metals, Co(II) complexes exhibit distinct electronic configurations, influencing their coordination behavior, reactivity, and biological interactions. Unlike redox-active metals such as Cu(II) or Fe(III), which may cause oxidative damage in biological systems, Co(II) offers more controlled reactivity while maintaining therapeutic potential [11, 12]. These characteristics make them compelling targets in drug development. Nevertheless, further investigation into their biological interactions and pharmacokinetic properties is necessary to fully understand their therapeutic potential.

Schiff bases are extensively utilized as ligands in coordination chemistry due to their strong metal-chelating ability, structural versatility, and biological significance [13-15]. Notably, the incorporation of oxime (-C=NOH) and o-vanillin moieties into Schiff bases ligands has been shown to enhance their biological activity. The oxime functional group contributes to improved antioxidant properties and stronger interactions with biological targets, while o-vanillin enhances solubility and bioavailability [16]. These features make Schiff base ligands incorporating oxime and o-vanillin particularly valuable for the development of bioactive metal complexes with potential therapeutic applications.

A critical aspect of evaluating the drug-like potential of metal complexes is understanding their interactions with biological macromolecules, such as bovine serum albumin (BSA). BSA is widely utilized as a model protein in drug-protein interaction studies, as it plays a crucial role in drug bioavailability, stability, and systemic distribution [17-19]. The binding affinity of a drug candidate with BSA is a key determinant of its pharmacokinetic behavior, influencing circulation time and therapeutic efficacy [20]. Strong interactions with BSA can prolong the drug's half-life, enhancing stability and controlled release, whereas weak binding may result in rapid clearance and reduced bioavailability

In addition to BSA binding studies, *in silico* ADME (absorption, distribution, metabolism, and excretion) predictions offer valuable insights into the pharmacokinetic properties of drug candidates. These computational approaches facilitate the early identification of promising compounds by predicting drug-like behavior, thereby reducing the reliance on resource-intensive experimental evaluations [21-24].

In this study, essential ADME parameters-including molecular weight, topological polar surface area (TPSA), number of hydrogen bond donors/acceptors, gastrointestinal absorption, and blood-brain barrier (BBB) permeability were analyzed to assess the drug-likeness and bioavailability of the synthesized complexes.

While several studies have investigated Schiff base metal complexes, this work presents a new Co(II) complex containing a Schiff base ligand derived from o-vanillin. The unique combination of oxime and o-vanillin moieties within the same ligand framework, along with the integration of BSA binding and *in silico* ADME analyses, distinguishes this study by offering a more comprehensive evaluation of the complex's pharmacokinetic and therapeutic potential.

The present study aims to synthesize and characterize a new Schiff base ligand and its Co(II) complex. The structural properties of ligand and Co(II) complex were investigated using spectroscopic techniques, and their biological potential was assessed through BSA binding studies and *in silico* ADME predictions. This comprehensive approach provides a deeper understanding of the pharmacokinetic and therapeutic potential of Co(II) complexes and contributes valuable knowledge to the field of metal-based drug development.

2. Experimental

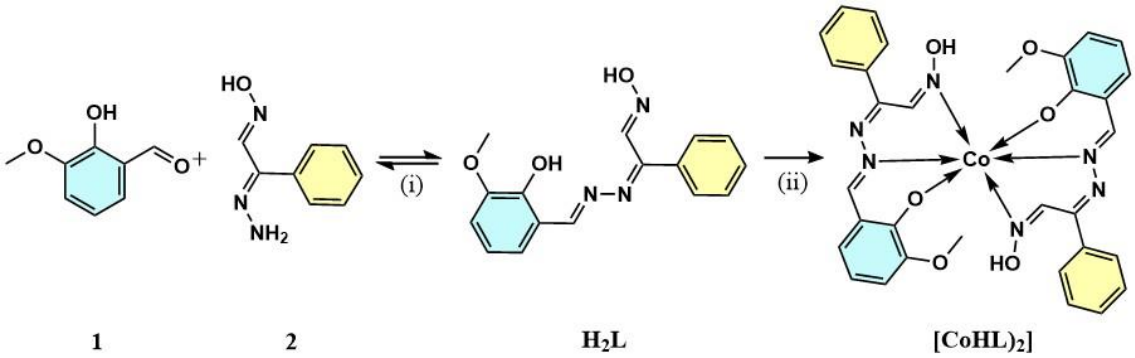
2.1. Materials and Measurements

All reagents were purchased from Merck/Sigma with a purity of at least 95% and were used without further purification unless otherwise stated. The supplementary file contains detailed descriptions of the materials, instrumentation, and experimental techniques used in the BSA binding studies. Isonitrosophenylhydrazine (2) was synthesized according to previously reported methods [25, 26].

2.2. Synthesis and Characterization

2.2.1. Synthesis of ligand

The ligand was synthesized according to the previously reported methods in literature [27, 28]. Briefly, a solution of isonitrosophenyl hydrazine (10 mmol, 1 eq) in 5 mL of methanol was added to a warm solution of o-vanillin (10 mmol, 1 eq) in 10 mL of a dichloromethane/methanol (9:1) solvent system. The reaction was conducted under reflux for 24 h in the presence of ca. acetic acid (AcOH). The reaction progress was monitored using thin-layer chromatography (TLC) with a ethyl acetate/hexane (3:7) solvent mixture. Upon completion, the reaction mixture was filtered, and the solvent was evaporated at room temperature, yielding the final product as a yellow solid (Scheme 1).



Scheme 1. Synthesis of ligand and Co(II) complex. (i) DCM/MeOH, 24 h, 65 °C, reflux, ca. AcOH. (ii) DCM/MeOH, RT, 24 h, reflux.

(1E,2Z)-2-(((E)-2-hydroxy-3-methoxybenzylidene)hydrazineylidene)-2-phenylacetaldehyde oxime (H_2L): Yield: 73%, m.p.: 146-148 °C. UV–Vis (DMF, nm) λ_{max}: 262, 319. IR (ATR, cm^{-1}): ν=3138-3040 (OH/NOH), 1608 (C=N), 1251 (C-O). ^1H NMR (400 MHz, DMSO-d_6, ppm): δ= 12.27 (s, 1H, Ar-OH), 12.23 (s, 1H, Ar-OH), 10.85 (s, 1H, NOH), 10.58 (s, 1H, NOH), 8.87 (d, *J* = 7.4 Hz, 2H, Ar-H), 8.66 (s, 1H, NCH), 8.11 (s, 1H, NCH), 7.84 – 7.77 (m, 2H, Ar-H), 7.54 – 7.43 (m, 6H, Ar-H), 7.40 – 7.29 (m, 3H, Ar-H), 7.18 – 7.10 (m, 2H, Ar-H), 7.07 (d, *J* = 7.9 Hz, 1H, Ar-H), 6.90 (m, 2H, Ar-H), 3.85 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, DMSO-d_6): δ= 165.25, 164.41, 161.03, 160.28, 150.11 (*C*OH), 149.28 (*C*OH), 148.85 (*C*OCH3), 148.48 (*C*OCH3), 148.22 (*C*NOH), 143.43 (*C*NOH), 135.64, 133.55, 130.72, 129.72, 129.42, 129.03, 128.49, 128.39, 123.89, 121.85, 119.83, 119.58, 119.45, 118.30, 115.90, 115.64, 56.38 (CH_3), 56.09 (CH_3). Elemental analysis (%) calcd. for $C_{16}H_{15}N_3O_3$, C 64.64, H 5.09, N 14.13; found: C 64.60, H 5.11, N 14.13. MALDI-TOF-MS (*m/z*): calculated for $C_{16}H_{15}N_3O_3$=297.11 $[M]^+$, found=298.64 $[M+H]^+$.

2.2.2. Synthesis of Co(II) complex

The Co(II) complex was synthesized following a reported procedure [29, 30]. A solution of cobalt(II) acetate monohydrate (5 mmol, 1 eq) in methanol (10 mL) was added dropwise into a solution of ligand (10 mmol, 2 eq) in dichloromethane (5 mL). The reaction mixture was stirred at room temperature (RT) for 24 hours, then filtered, and the solvent was evaporated under ambient conditions to obtain the final product as dark brown solid. The precipitate was treated with diethyl ether, water, and then dried.

Bis[(E)-2-(2-hydroxy-3-methoxybenzylidene)hydrazineylidene]-2-phenylacetaldehyde)cobalt(II) ($[Co(HL)_2]$): Dark brown solid; Yield: 78%; m.p: 221-223°C. B.M.: 4.90. UV–Vis (DMF, nm) λ_{max}: 272, 308, 500. IR (ATR, cm^{-1}): ν=3059 (NOH), 1598 (C=N), 1242-1211 (C-O). Elemental analysis (%) calcd. for $C_{32}H_{24}CoN_6O_6$, C 58.99, H 4.33, N 12.90; found: C 58.98, H 4.33, N 12.9. MALDI-TOF-MS (m/z): calculated for $C_{32}H_{24}CoN_6O_6$=651.14 $[M]^+$, found=651.959 $[M+H]^+$.

2.3. BSA Binding Studies

BSA-compound interactions were investigated using UV-Vis and fluorescence spectroscopy, as described in earlier studies [31, 32]. Detailed procedures for the BSA interaction studies, as well as the calculations of binding data from fluorescence measurements of ligand and Co(II) complex, are provided in the ESI. Experiments were conducted in triplicate.

2.4. *In Silico* Studies

Parameters such as absorption, distribution, metabolism, excretion (ADME) define the essential characteristics a drug molecule should possess. The various ADME properties including TPSA, MW, no. of rotatable bonds/hydrogen bond donors/hydrogen bond acceptors, Log P, Log S, compatibility to Lipinski rule, GI absorption, BBB permeant, skin permeant, bioavailability, synthetic accessibility were predicted using the SwissADME online tool [33].

3. Results and Discussion

3.1. Spectral Characterization

3.1.1. Infrared Spectroscopy

To elucidate the coordination behavior of the Co(II) ion with the ligand, the infrared (IR) spectra of the ligand (Figure S1) and its Co(II) complex (Figure S2) were compared. In the IR spectrum of the ligand, a characteristic phenolic O-H stretching band appeared at 3138 cm^{-1}. The disappearance of this band upon complexation suggests deprotonation of the phenolic group and its subsequent involvement in metal coordination [34]. Additionally, coordination through the azomethine nitrogen is supported by the shift in the C=N stretching frequency, which moved from 1608 cm^{-1} in the free ligand to 1598 cm^{-1} in the Co(II) complex. This downward shift reflects a reduction in bond order due to electron donation from the nitrogen to the metal center, further confirming the participation of the azomethine nitrogen in coordination [35-38].

3.1.2. UV-Visible Spectroscopy

The electronic absorption spectra of the ligand and its Co(II) complex were recorded in DMF solution over the wavelength range of 200–800 nm at room temperature

(Figure S3). The ligand exhibited characteristic π-π* and n-π* electronic transitions at 262 nm and 319 nm, respectively, corresponding to the transitions within the conjugated aromatic system and the lone pair electron excitation of the azomethine (C=N) group [39].

Upon complexation with the Co(II) ion, notable shifts in absorption bands were observed. The Co(II) complex displayed absorption bands at 272 nm and 308 nm, attributed to ligand-centered transitions, indicative of metal-ligand electronic interactions. Additionally, a broad absorption band was observed around 500 nm, characteristic of d-d transitions within the Co(II) ion's d^7 electronic configuration in an octahedral geometry [40]. The presence of these d-d transitions strongly supports the coordination of the ligand to the Co(II) center, further confirming the formation of the metal complex.

3.1.3. Nuclear Magnetic Resonance Spectroscopy

The ^{1}H NMR and ^{13}C NMR spectrum of the ligand was recorded in DMSO-d_6 (Figure 1a and b) and revealed distinct proton signals corresponding to the *E* and *Z* isomers of the ligand.

In the aromatic region, additional signals corresponding to protons on the benzene rings were identified between 6.90 and 8.66 ppm, further confirming the structural integrity of the ligand. The hydroxyl (OH) proton signals were observed at 12.27 ppm (singlet) for the *E* isomer and 12.23 ppm (singlet) for the *Z* isomer, with slight shifts attributed to variations in hydrogen bonding [26]. Notably, the signals at 10.85 ppm and 10.58 ppm were assigned to the oxime (-NOH) protons of the *E* and *Z* isomers, respectively [41]. These singlets reflect the chemical environment of the oxime functional groups and their distinct interactions within each isomeric configuration. Additionally, the methoxy group protons displayed distinct chemical shifts for each isomer, appearing as a singlet at 3.74 ppm for the *E* isomer and 3.85 ppm for the *Z* isomer [42].

The ^{13}C NMR spectrum of ligand reveals the presence of *E* and *Z* isomers, as evidenced by the duplication of key resonances. These distinct chemical shifts confirm the coexistence of both isomeric forms, similar to the observations in the ^{1}H NMR spectrum.

The aromatic region displays multiple signals between 115.64 and 150.11 ppm, corresponding to the benzene ring carbons of both isomers. The downfield shifts at 160.28, 161.03, 164.41, and 165.25 ppm are attributed to C=N and 149.28, 150.11 ppm are attributed to C-OH functional groups, with minor variations between the two isomers due to electronic and steric effects.

The oxime (-C=NOH) carbon resonates at 148.22 ppm for the *E* isomer and 143.43 ppm for the *Z* isomer, reflecting the differences in hydrogen bonding and electronic distribution around this functional group.

A notable feature of the spectrum is the differentiation of the methoxy (-OCH_3) group between the two isomers. The signal appears at 56.09 ppm for the *Z* isomer and 56.38 ppm for the *E* isomer, aligning well with the proton NMR data, where distinct chemical shifts were observed for these groups [43].

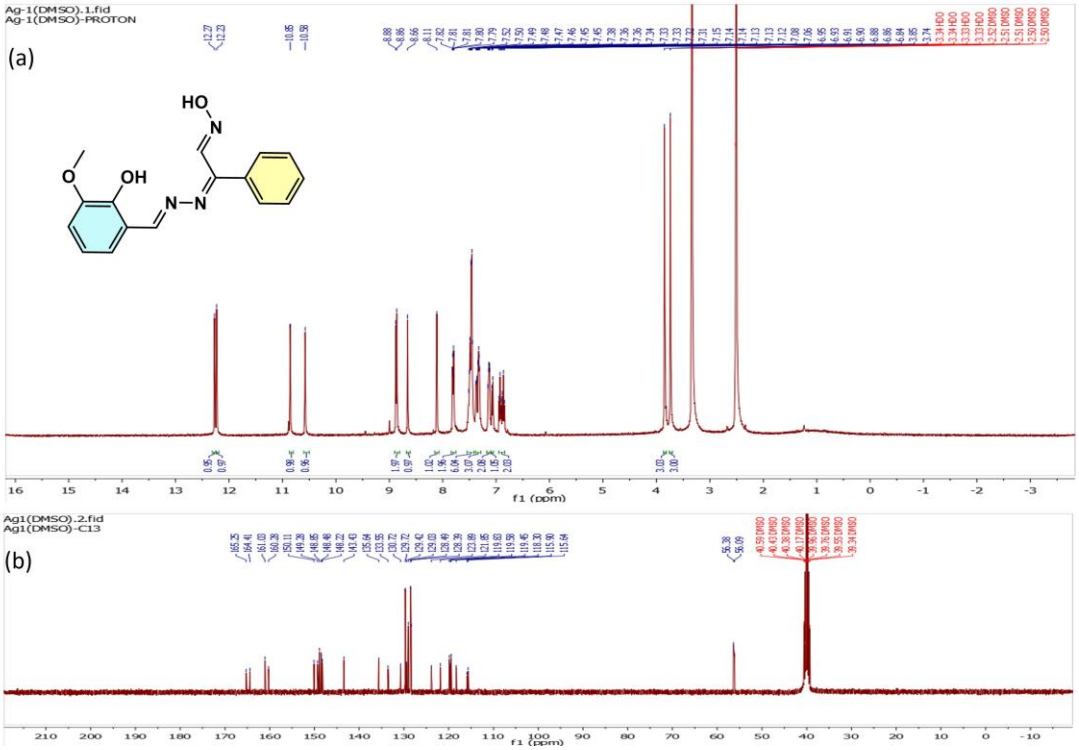


Figure 1. (a) ^{1}H NMR and (b) ^{13}C NMR spectra of Schiff base ligand

Spectroscopic features clearly support the presence of both *E* and *Z* isomers in solution. Analysis of the integrals for the *E* and *Z* signals indicated an approximate equilibrium ratio of 1:1.

3.1.4. Mass Spectral Analysis

The MALDI-TOF-MS spectra of the ligand and its Co(II) complex are presented in Figures S4 and S5, respectively. For the ligand, the spectrum exhibits a prominent molecular ion peak at *m/z*=298.640, corresponding to the $[M+H]^+$ ion, which is in good agreement with the calculated value of 297.110.

In contrast, the MALDI-TOF-MS spectrum of the Co(II) complex displays a molecular ion peak at *m/z*=651.959, attributed to the $[M+H]^+$ ion. This experimental value closely matches the calculated mass of 651.140, further supporting the successful coordination of the ligand with the Co(II) ion.

The observed shifts in the molecular ion peaks between the ligand and the Co(II) complex provide clear evidence of complex formation, consistent with the proposed stoichiometry.

3.1.5. Magnetic Susceptibility Measurements

Room-temperature magnetic susceptibility measurements confirmed the paramagnetic nature of the Co(II) complex. The observed magnetic moment was determined to be 4.90 B.M., consistent with the expected range for mononuclear Co(II) complexes containing a single Co(II) ion in a d^7 electronic configuration. This value aligns well with a high-spin configuration, characteristic of Co(II) complexes in an octahedral coordination geometry [44].

3.2. BSA Binding Studies

Bovine serum albumin (BSA) is frequently employed in pharmaceutical research to investigate drug interactions due to its structural similarity to human serum albumin [45, 46].

Fluorescence quenching and UV-Vis absorption spectroscopy were employed to elucidate the interaction mechanism between compounds and BSA. In particular, the UV-Vis absorption spectrum of BSA provides crucial insights into the quenching mechanism, enabling the differentiation between static and dynamic quenching based on spectral variations upon complex binding. In dynamic quenching, no changes in the absorption spectra are usually observed. However, in static quenching, where a ground-state complex forms between the quencher and BSA, alterations in the absorption spectra are expected [47].

Upon the addition of both ligand and Co(II) complex, the UV-Vis absorption spectrum of BSA exhibited an increase in absorbance accompanied by a slight blue shift (Δλ≈2 nm). This spectral change suggests a potential static quenching mechanism, indicating the formation of a ground-state complex between BSA and compounds (Figure 2).

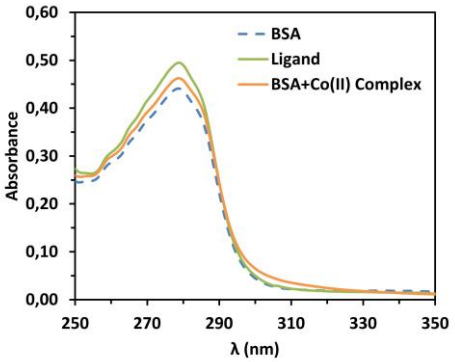


Figure 2. UV-Vis. spectra of BSA (10 μM) in the absence and presence of ligand and Co(II) complex (10 μM).

Fluorescence spectroscopy is a widely recognized technique employed to explore the binding affinities and interaction mechanisms of different compounds with BSA. The fluorescence properties of BSA are largely influenced by tryptophan and tyrosine residues, with tryptophan being particularly responsible for spectral changes associated with protein conformational shifts, denaturation, or substrate binding [48, 49].

In this study, BSA (5 μM) was titrated with increasing concentrations (0-10 μM) of the ligand (H_2L) and its Co(II) complex to assess their binding affinity and quenching behavior. Upon excitation at 280 nm, BSA exhibited a strong emission band centered at 345 nm. The addition of both compounds caused a concentration-dependent decrease in fluorescence intensity, with a quenching of 25.68% observed for the free ligand and 46.87% for the Co(II) complex (Figure 3). This suggests that both compounds interact with BSA, with the Co(II) complex inducing significantly stronger quenching, indicative of enhanced binding affinity after metal coordination.

The quenching constant (K_{sv}) for the ligand and Co(II) complex was obtained using the Stern-Volmer equation, based on the I_0/I versus [Q] plot (Figure 6S and 7S), to evaluate the interaction strength and quenching mechanism with bovine serum albumin (BSA). The Stern-Volmer quenching constant (K_{sv}) was calculated as 3.02×10^4 M^{-1} for the ligand and 7.81×10^4 M^{-1} for the Co(II) complex (Table 1), clearly demonstrating the increased quenching efficiency of the complex.

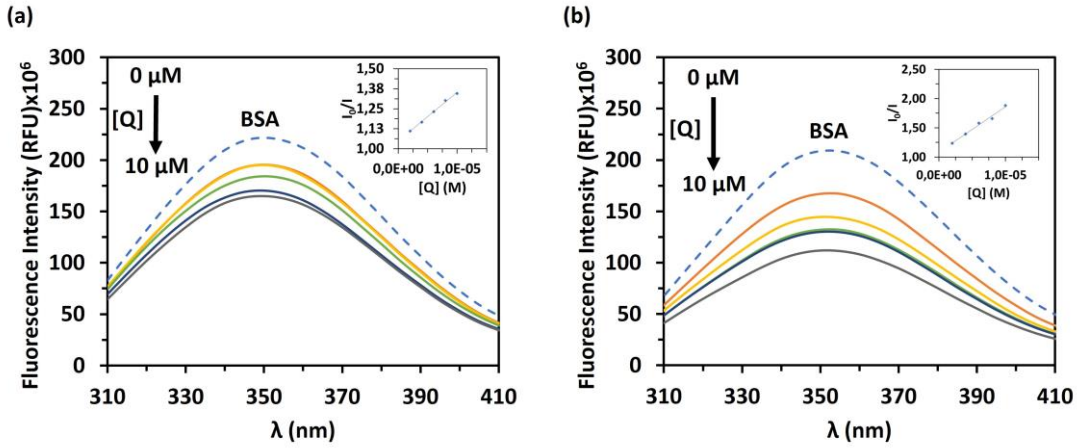


Figure 3. Emission spectra of BSA (5 µM) in presence of increasing concentrations of (a) ligand and (b) Co(II) complex. Insets show Stern–Volmer plot of I_0/I versus [Q].

Additionally, the binding constant (K_{bin}) was calculated using the Scatchard equation, $\log[(I_0 - I)/I] = \log K_{bin} + n\log[Q]$, where K_{bin} indicates the binding constant and n represents the number of binding sites. The Co(II) complex showed a significantly higher binding constant (8.74×10^3 M^{-1}) compared to the free ligand (1.36×10^3 M^{-1}), highlighting the role of metal coordination in enhancing protein-binding affinity. From the Scatchard analysis, the calculated n values (0.72 for ligand, 0.80 for Co(II) complex) indicates that the compounds bind to a single site on the BSA molecule [50].

To further evaluate the binding affinity of the synthesized Co(II) complex with BSA, its binding constant (K_{bin}=8.74×10^3 M^{-1}) was compared with similar Co(II) Schiff base complexes reported in the literature. For instance, a Co(II) complex derived from a tetradentate o-vanillin-based Schiff base exhibited a binding constant of K_{bin}=3.77×10^4 M^{-1}, indicating a slightly higher BSA affinity than our complex [51].

Thermodynamic analysis provided additional insight into the interaction strength. The Gibbs free energy (ΔG) for the Co(II) complex-BSA interaction was calculated as -22.49 $kJ.mol^{-1}$, indicating a spontaneous and thermodynamically favorable binding process. For the ligand, ΔG was -17.88 $kJ.mol^{-1}$, which also indicates spontaneity.

Table 1. The BSA binding data for ligand and Co(II) complex.

	H_2L	$[Co(HL)_2]$
K_{sv} ($x10^4$ M^{-1})	3.02 ± 0.15	7.81 ± 0.17
K_{bin} ($x10^3$ M^{-1})	1.36 ± 0.11	8.74 ± 0.09
k_q ($x10^{12}$ $M^{-1}.s^{-1}$)	3.02 ± 0.15	7.81 ± 0.17
n	0.72	0.80
ΔG ($kJ.mol^{-1}$)	-17.88	-22.49

Additionally, the bimolecular quenching rate constants (k_q), calculated using the known lifetime of BSA ($\tau_0=10^{-8}$ s), were 3.02×10^{12} $M^{-1}.s^{-1}$ and 7.81×10^{12} $M^{-1}.s^{-1}$ for the ligand and complex, respectively. These values notably exceed the maximum diffusion-controlled quenching constant (~2.0×10^{10} $M^{-1}.s^{-1}$), suggesting a static quenching mechanism, typically associated with complex formation in the ground state [52].

The quenching and binding constants demonstrate that coordination of the ligand to Co(II) significantly enhances its interaction with BSA in terms of both binding strength and quenching efficiency. The observed static quenching mechanism, coupled with favorable thermodynamic parameters, points to the formation of a stable compound–protein complex, which is crucial in understanding the potential pharmacokinetic behavior of the Co(II) complex in biological systems.

3.3. ADME Studies

The absorption, distribution, metabolism, and excretion (ADME) properties of the synthesized Co(II) complex were predicted using the SwissADME online tool [33], providing key insights into its pharmacokinetic profile (Table 2). The complex exhibits favorable physicochemical characteristics, with a molecular weight of 651.53 g/mol and a polar surface area (TPSA) of 151.54 $Å^2$, suggesting potential bioactivity. Despite two minor Lipinski rule violations, its moderate number of rotatable bonds (n=4) contributes to a relatively rigid molecular structure, which may enhance selective target binding while minimizing off-target interactions.

The metabolic stability of the Co(II) complex appears promising, as it does not significantly inhibit major cytochrome P450 enzymes, reducing the likelihood of drug-drug interactions—an essential factor in drug safety [53]. Additionally, although the complex exhibits low gastrointestinal (GI) absorption and does not cross the blood-brain barrier (BBB), these properties may be advantageous for targeting peripheral diseases, avoiding potential central nervous system side effects [54]. Notably, the complex is identified as a P-gp substrate, which may influence its bioavailability and cellular transport mechanisms.

Table 2. The pharmacokinetic properties of the Co(II) complex

	$[Co(HL)_2]$
Molecular weight (g/mol)	651.53
Number Heavy atoms	45
Rotatable bonds	4
H-Bond acceptors	8
H-Bond donors	2
Molar refractivity	188.78
TPSA ($Å^2$)	151.54
Log $P_{o/w}$	1.77
GI absorption	Low
BBB permeant	No
P-gp substrate	Yes
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (cm/s)	-6.37
Lipinski	No, 2 violations

4. Conclusions

In this study, a new Schiff base ligand containing an o-vanillin moiety and its Co(II) complex was successfully synthesized and characterized. The molecular structure of the Co(II) complex was confirmed through a combination of spectroscopic techniques, including UV-Vis, FTIR, magnetic susceptibility and MALDI-TOF-MS, which verified the octahedral coordination environment with two ligand molecules bound to the Co(II) ion. The NMR spectra provided clear evidence of the ligand's tautomeric equilibrium, while IR and UV-Vis data supported successful metal coordination.

BSA binding studies revealed moderate interaction for the Co(II) complex, with a binding constant (K_{bin}=8.74 x 10^3 M^{-1}) and a Gibbs free energy (ΔG=-22.49 kJ.mol^{-1}), indicating a spontaneous static quenching mechanism. The free ligand exhibited a significantly lower binding constant (K_{bin}=1.36 x 10^3 M^{-1}), suggesting that metal coordination substantially enhances the compound's protein-binding affinity. This increase in affinity can be attributed to improved structural rigidity and the overall electronic contribution of the metal center.

When compared with literature-reported Co(II) Schiff base complexes [11, 12], our complex falls well within the expected and pharmacologically favorable range. This moderate binding strength is beneficial, as it provides a balance between sufficient protein interaction and controlled release, both of which are desirable features for therapeutic agents.

In silico ADME predictions highlighted favorable physicochemical properties but indicated limited gastrointestinal absorption and blood-brain barrier impermeability. While this restricts its potential for central nervous system applications, future modifications such as prodrug strategies or nanoparticle-based delivery systems could enhance bioavailability and facilitate targeted therapeutic applications.

Based on the data obtained throughout the study, its concluded that the synthesized Co(II) complex demonstrates promising structural, and biological properties, making it a potential candidate for therapeutic applications, particularly in the treatment of cancer, due to its favorable protein-binding properties, as well as its drug-like pharmacokinetic profile. Further studies are needed to validate the biological activity of this complex. *In vitro* cytotoxicity assays will be conducted to evaluate its potential anticancer properties, followed by *in vivo* pharmacokinetic studies to determine its metabolic stability and biodistribution. These investigations will be essential for assessing the therapeutic viability of the Co(II) complex and guiding its potential biomedical applications.

5. Acknowledgment

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6. References

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