



Synthesis, Characterization and DNA-Binding of Cobalt(II) Complex with 3-Benzyl-4-(*p*-hydroxy-*m*-methoxybenzylidenamino)-1*H*-1,2,4-triazole-5-one

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In this work, the synthesis, characterization and DNA-binding of cobalt(II) complex with 3-benzyl-4-(*p*-hydroxy-*m*-methoxybenzylidenamino)-1*H*-1,2,4-triazole-5-one has been reported. The newly prepared compound was characterized by elemental analyses, IR, UV-vis, ¹H NMR spectra. The interaction of the Co(II) complex with cat DNA was investigated by UV absorption spectroscopy and viscosity measurements. The binding constant of the complex was determined using absorption measurement and found to be $0.98 \times 10^4 \text{ M}^{-1}$. Results suggest that the complex binds to DNA *via* an intercalative binding mode.

Key Words: Co(II) complex, Schiff base, DNA-binding.

INTRODUCTION

The binding metal complexes to DNA have been comprehensively studied as DNA footprinting and cleaving agents and probable anticancer drugs¹⁻³. In these kind of compounds, metal or ligand may be differ to enable the applications. DNA molecule has several types of intercalation sites where binding of metal compounds can take place between intercalation, in groove and on the outside of the helix⁴. Plenty biological tests have also proved that DNA is the first intracellular target of anticancer drugs because of the interaction between small metal compounds and DNA, which can lead to DNA damage in cancer cells, preventing the division of cancer cells and causing cell death⁵⁻⁷.

Schiff bases perform important role in bioinorganic chemistry since they have significant biological activities. Some metal complexes of Schiff bases can cause to DNA damage, cleavage⁸⁻¹². Schiff bases the dependence of their mode of binding with transition metal ions in the living system have been of remarkable interest¹³⁻¹⁷. The transition metal compounds of Schiff bases have been reported to behave for enzyme inhibitors¹⁸ and are beneficial because of their pharmacological applications¹⁹⁻²¹. Before some work suggests that 3-(*p*-chlorobenzyl)-4-(*p*-hydroxy-*m*-methoxybenzylidenamino)-1*H*-1,2,4-triazol-5-one shows antibacterial, antioxidant and analgesic activity²², but no metal complexes of such compound have been reported in the past which can have better pharmaceutical effect potential. Therefore, study of the metal complexes of this schiff base is important to discover the potential new drug.

In the present study, 3-benzyl-4-(*p*-hydroxy-*m*-methoxybenzylidenamino)-1*H*-1,2,4-triazole-5-one compound was synthesized and characterized and its cobalt(II) complex was synthesized. In addition to this study, the DNA binding properties with the complex was studied.

EXPERIMENTAL

All solvents and reagents were provided commercially and used without further purification. Cat-DNA was obtained from Veterinary School of Medicine at Kafkas University. The concentration of DNA was determined spectrophotometrically using a molar absorptivity of $6600 \text{ M}^{-1} \text{ cm}^{-1}$ (260 nm)¹⁴. Infrared spectra ($4000\text{-}400 \text{ cm}^{-1}$) were determined with KBr disks on a Thermo Mattson FTIR spectrometer. The UV-visible spectra were recorded on a Varian Cary 100 UV-vis spectrophotometer. ¹H NMR spectra were measured on a Varian VR300-MHz spectrometer, using TMS as a reference.

Preparation of the ligand of 3-benzyl-4-(*p*-hydroxy-*m*-methoxybenzylidenamino)-1*H*-1,2,4-triazole-5-one compound: The ligand (Fig. 1) was prepared according to the literature²². 4 g of 4-amino-3-(*p*-chlorobenzyl)-1*H*-1,2,4-triazole-5-one with 0.80 g of 4-hydroxy-3-methoxybenzaldehyde were boiled in 30 mL ethanol under condenser for 2 h. Then, it was left for vapourization of the solvent. After obtained solid yield was crystallized in the alcohol, the yield was filtered and white solid (m.p. 268 °C) left to dry in drying oven, ¹H NMR (300 MHz, DMSO) (ppm): 3.8(-OCH₃), 4.01 (-CH₂-), 7.01-7.34 (Ar-H), 9.75 (N=CH), 9.75 (OH) and 11.33 (NH). Anal. calcd. (%) for C₁₇H₁₆N₄O₃: C, 62.95; H, 4.97; N 17.29. Found. (%):

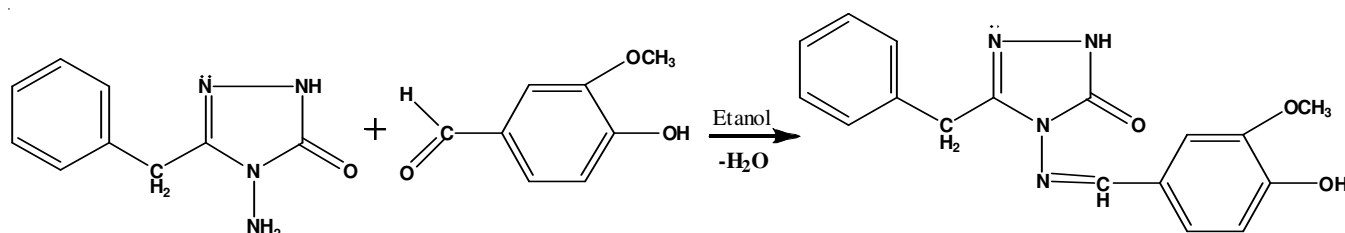


Fig. 1. Scheme of the synthesis of the ligand

C, 62.83; H, 4.80; N, 17.27. IR (KBr, ν_{\max} , cm^{-1}): (C=O): 1707, (C=N): 1678, (N-H): 3256, (O-H): 3400.

Preparation of 3-benzyl-4-(*p*-hydroxy-*m*-methoxybenzylideneamino)-1H-1,2,4-triazole-5-one cobalt(II) compound: 5 mL NaOH was added to 0.410 g of (1.23 mmol) of 3-benzyl-4-(*p*-hydroxy-*m*-methoxybenzylidene amino)-1H-1,2,4-triazole-5-one compound. The solution was stirred up 0.5 h at 60 °C. 0.36 g $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ solution was added to the mixture. The colour of solution turned to violet. The reaction mixture were stirred under the condenser for 2 h and the mixture was kept at room temperature. The mixture were filtered to remove any insoluble residues and then stirring was continued for 24 h at room temperature. A violet precipitate of the cobalt(II) complex (Fig. 1), was separated from the solution by filtration, purified by washing several times with ethanol and dried for 3 h under vacuum (m.p. 292 °C, yield 65 %. Anal. calcd (found) (%) for the Co(II) complex $\text{C}_{34}\text{H}_{34}\text{N}_8\text{O}_8\text{Co}$: C, 55.06 (55.03); H, 4.62 (4.48); N, 15.11 (14.76). ^1H NMR (300 MHz, DMSO) (ppm): 3.8 (-OCH₃), 4.06 (-CH₂-), 7.01-7.34 (Ar-H), 9.45 (N=CH), 11.95 (NH). IR (KBr, ν_{\max} , cm^{-1}): (C=O): 1707, (C=N): 1608, (N-H): 3256, (Co-O): 579.

DNA-Binding experiments: The UV-vis absorbance experiment showed that the DNA solution in *tris*-HCl buffer at pH 7.1 at 260 and 280 nm was adequately free of protein²³. The concentration of DNA was determined by measuring the UV absorption at 260 nm as $6600 \text{ M}^{-1} \text{ cm}^{-1}$ ²⁴. The intrinsic binding constant (K_b) for the interaction of the cobalt(II) complex with cat DNA was calculated by absorption spectra data.

Viscosity measurements: Viscosity experiment was performed on an Ubbelodhe viscometer, which was submerged in a thermostated water bath maintained at 30.0 ± 0.1 °C. The viscosity experiment was conducted for the complex (1-10 μM) and cobalt(II) complex was imbeded the cat DNA solution (10 μM) present in the viscometer. Data were shown as $(\eta/\eta_0)^{1/3}$

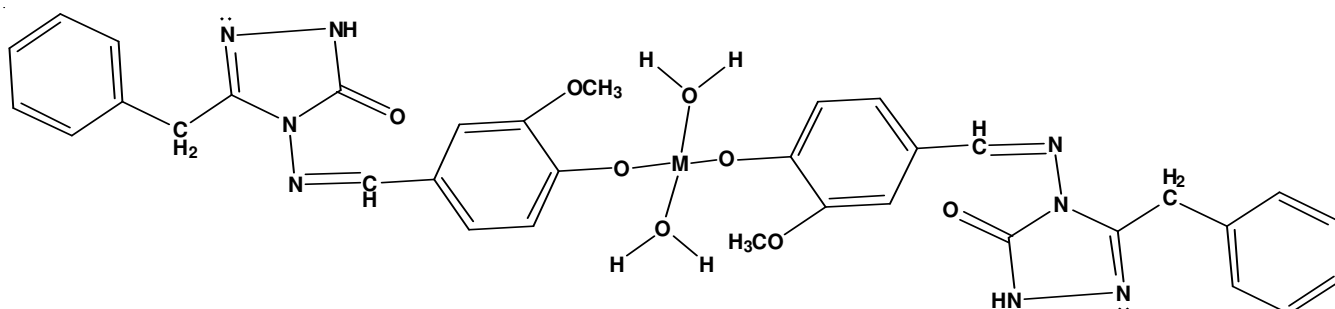
versus the ratio of the concentration of the cobalt(II) complex to the DNA, where σ represents the viscosity of the DNA in presence of the metal complex and η_0 represents the viscosity of cat DNA in the absent of the complex. Viscosity values were assessed from the observed flow time of the DNA containing solutions corrected from the flow time of buffer alone (t_0), $\eta = t - t_0$ ²⁵.

RESULTS AND DISCUSSION

Experimental evidences show that the Schiff base formed a mononuclear cobalt(II) complex, the probable structure of the complex is shown in Fig. 2. This presume is in agreement with elemental analysis and IR data. The results suggest that the composition of the cobalt(II) complex is $\text{C}_{34}\text{H}_{38}\text{N}_8\text{O}_{12}\text{Co}$. The complex is stable and soluble in DMSO, DMF, methanol and ethanol.

The IR spectrum of the free ligand and the complex show bands at IR (KBr, ν_{\max} , cm^{-1}) for ligand: (C=O): 1707, (C=N): 1678, (N-H): 3256, (O-H): 3400 and (C=O) for complexes: 1707, (C=N): 1608, (N-H): 3256, (Co-O): 579, respectively. The band corresponding to (O-H) at 3400 cm^{-1} indicates that the complex contains water molecules that is in accordance with the results of elemental analysis.

The application of electronic absorption spectroscopy in DNA-binding studies is one of the most useful techniques²⁶. The absorption spectra of the ligand (Fig. 3) and cobalt(II) complex (Fig. 4) in the absence and presence of cat DNA at a constant concentration of complex is given in Fig. 5. The study of the electronic spectra in the UV for cobalt(II) complex and the ligand were performed in the buffer solution. The electronic spectra of ligand had a strong bands at 304 nm and at 310 nm. There are two bands at 280 and 310 nm for $\text{C}_{34}\text{H}_{34}\text{N}_8\text{O}_8\text{Co}$ complex. These changes show that complex is formed. The spectroscopic changes suggest that the complex has interaction with DNA. The intrinsic binding constants K_b of cobalt(II)

Fig. 2. Structure of 3-benzyl-4-(*p*-hydroxy-*m*-methoxybenzylideneamino)-1H-1,2,4-triazole-5-one cobalt(II) compound (M=Co)

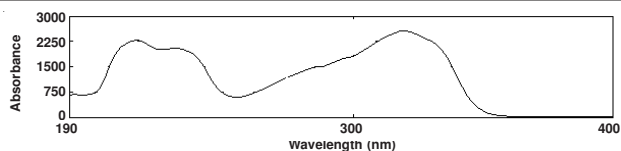


Fig. 3. Electronic spectra of $C_{17}H_{16}N_4O_3$ in the absence of the cobalt(II) ion

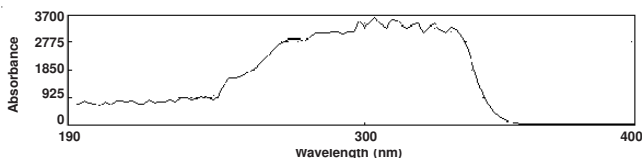


Fig. 4. Electronic spectra of $C_{34}H_{34}N_8O_8Co$ in the absence of the DNA

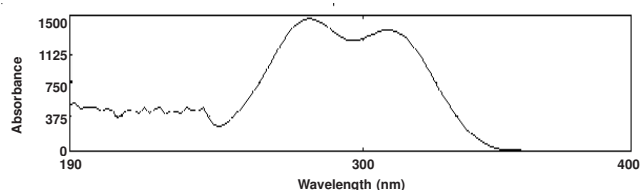


Fig. 5. Electronic spectra of $C_{34}H_{34}N_8O_8Co$ in the presence of the DNA. The arrow indicates the absorbance changes upon increasing DNA concentration

complex was $0.98 \times 10^4 M^{-1}$. The result indicate that the binding strength of cobalt(II) complex^{27,28}. This result indicates that cobalt(II) compound binds the DNA as intercalative^{29,30}.

Viscometric method is an important tool in explaining the mode of interaction of small molecules with DNA. Mostly, intercalation lead to an increase in the viscosity of DNA solution because of the lengthening of DNA helix as the base pairs are enlarged to coordinate the binding molecule³¹. The plot of relative specific viscosity ((η/η_0) versus $[Co(II) \text{ complex}]/[DNA]$ is shown in Fig. 6.

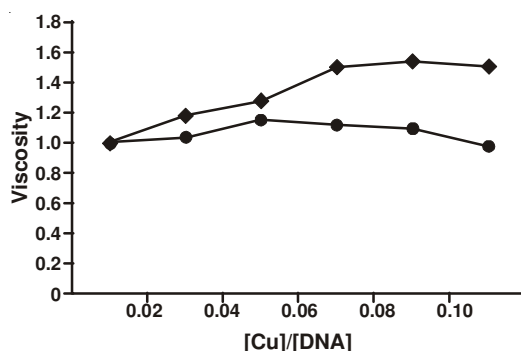


Fig. 6. Impact of increasing concentration of the Co complex (◆) on the relative viscosity of DNA (●) at 30.0 °C

The plot determines positive change in η/η_0 with increasing concentration of Co(II) complex. Such a behaviour is an evidence of electrostatic interaction, that may leads to conglomerate of DNA. The conglomeration decreases the number of separately moving DNA molecules which results in decreasing of the

solution viscosity. The results show that the complex increases the DNA viscosity, which is coherent with DNA groove binding that is also known to increases DNA viscosity³².

Conclusion

The cobalt(II) complex of 3-benzyl-4-(*p*-hydroxy-*m*-methoxybenzylideneamino)-1*H*-1,2,4-triazole-5-one has been prepared and characterized. The DNA-binding of cobalt complex was investigated by UV-vis absorption, viscosity measurement. Experimental results show that the complex can bond to cat DNA with the mode of intercalative binding.

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