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Research Paper

Synthesis and Characterization of Chromium (III) Complexes of 4(3*H*)-quinazolinone derived Schiff base: Antimicrobial and DNA interaction Studies

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Abstract- *The condensation of 2-benzofuran carboxaldehyde with 3-amino-2-methyl-4(3H)quinazolinone has lead to the isolation of quinolin-4(3H)-one derived Schiff base. The chromium (III) complexes of the ligand were also prepared. Both free ligand and its chromium (III) complexes were characterized by elemental analyses, spectral methods (IR, ^{1}H-NMR, MS and UV–Vis), magnetic and molar conductance measurements, and thermal studies. The bidentate ligand coordinated to the chromium (III) ion through the lactum oxygen and the azomethine nitrogen of the ligand. All of the compounds were investigated for their antimicrobial activities against the Gram-positive and Gram-negative bacteria and fungi. In addition, the DNA binding and nuclease activities of Chromium (III) complexes were performed.*

Keywords: Quinolin-4(3*H*)-one, antimicrobial activity, DNA binding studies, nuclease activity.

Introduction

Schiff bases of azomethine nitrogen donor heterocyclic ligands are well known due to their wide range of applications in pharmaceutical and industrial fields. [1] Transition metal complexes of Schiff bases have attracted much attention due to their potent biological activities such as antifungal, antibacterial, anticancer and herbicidal applications [2-5]. Investigations on the interaction between transition metal complexes and DNA have created interests due to their importance in cancer therapy and molecular biology[6].

Among them, Schiff base metal complex is a kind of attractive reagent due to their special activities in pharmacology and physiology [7-9]. During the last decade, transition metal complexes of Schiff base derived from 2-pyridinecarboxaldehyde and different amines have received considerable attention on the part of synthetic and biological activities of DNA binding and cleavage with high sequence and structure selectivity[10-12]. In this communication, we describe the synthesis, antimicrobial, DNA binding and cleavage abilities of Cr (III) complexes with a Schiff base ligand derived from3-amino-2-methyl-4(*3H*)-quinazolinone.

Material and Methods

Chemicals used: 3-amino-2-methyl-4(*3H*)-quinazolinone and 2-benzofuran carboxaldehyde was obtained from Aldrich. Chromium(II) salts and solvents were commercially available of high purity. Calf thymus DNA (CT-DNA) purchased from Genie Laboratories, Bangalore.

An Elemental analysis was carried out using Perkin-Elmer 240 elemental analyzer. Infrared spectra were recorded with Shimadzu FT-IR 8300 spectrophotometer from 4000-400 cm^{-1} using nujol mulls technique. Magnetic susceptibility measurements at room temperature were done using Sherwood scientific MXI model Gouy magnetic balance. The UV-Visble spectra were recorded on Hitachi-3900 spectrophotometer. Shimadzu TG-50H thermo analyzer was used to record simultaneous TGA and DTG curves in dynamic nitrogen atmosphere with a heating rate of 10 °C min^{-1}, in the temperature range 20-700 °C using platinum crucibles. ^{1}H-NMR spectra were recorded using Varian-400 MHz spectrometer using DMSO-d_6 as a solvent. Chemical shifts are reported in parts per million downfield from tetramethylsilane. EIMS were determined on ABS API-2000 mass spectrometer.

Synthesis of 3-(isobenzofuran-1-ylmethyleneamino)-2-methylquinazolin-4(*3H*)-one (L)

A 1:1 equimolar solution of 3-amino-2-methyl-4(*3H*)-quinazolinone (0.350 g, 2 mmol) and 2-benzofuran carboxaldehyde (0.263 g, 2 mmol) were mixed in 30 mL methanol and gently heated for 3 h with constant stirring. The characteristic yellow precipitate of Schiff base obtained by condensation was filtered and crystallized using ethanol.

Yield: 83%, IR (nujol mulls, cm^{-1}): 3088.4, 2924.5, 2161.8 (C-H), 1684.2 (C=O), 1597.0 (C=N), ^{1}H-NMR (400 MHz,

DMSO-d_6) δ: 2.72 (s, CH_3, 3H, C_8), 8.9 (s, CH, 1H, N=CH-), 7.4-8.2 (m, Ar-H, 8H, Aromatic protons), Mass (m/z): 265 [M^++1], Anal: Calcd. For $C_{15}H_{12}N_4O$: C 68.18, H 4.28, N 21.22, Found: C 68.27, H 4.3, N 21.32 %. Based on these data, the following molecular structure has been assigned to the Schiff base (Figure 1).

Synthesis of metal complexes

The Cr(III) complexes of Schiff base ligand were prepared in 1:2 [metal:ligand] and 1:1:1 [metal:ligand:1,10-phenanthroline] ratios.

To a 20 ml hot methanolic solution of metal chloride (0.170 g, 1 mmol), Schiff base ligand solution was added (L, 2 mmol) to obtain 1:2 complex and a methanolic solution of 1,10-phenanthroline (0.198 g, 1 mmol) was added slowly in the presence of 1 mmol of L with continuous stirring to obtain 1:1:1 complex. The resulting mixture was stirred under reflux for 4 h to obtain the precipitated complex. It was collected by filtration, washed with hot water, then diethyl ether and dried in air. The analytical and physical data were reported in Table 1.

Biological activity

Antimicrobial studies: The *in vitro* antimicrobial activity of Schiff base ligand and its Cr(III) complexes were determined by standardized disk-agar diffusion method[13] against the sensitive organisms *Staphylococcus aureus* as Gram positive and *Escherichia coli* as Gram negative bacteria and the fungi *Aspergillus niger* and *Fusararium oxysporum*. Chloramphenicol was used as a standard reference in the case of bacteria, while Griseofulvin was used as a standard for antifungal reference.

The tested compounds were dissolved in DMSO (no inhibition activity) to get concentration of 1 mg/mL. The test was performed on nutrient agar medium for antibacterial activity and Sabraoud dextrose agar medium for antifungal activity [14]. Sterile disks were soaked in test compounds and carefully placed on incubated agar surface. The petri dishes were incubated for 24 h at 37°C in the case of bacteria and for 48 h at 37°C in the case of fungi. Finally, the zone of inhibition was carefully measured.

DNA binding studies: All the experiments involving the interaction of complexes with CT-DNA were conducted in Tris buffer (10 mm Tris-HCl-50 mm NaCl buffer, pH 7.4). The concentration of the DNA used for binding experiments was determined by measuring the absorption intensity at 260 nm with molar extinction coefficient value 6600 M^{-1} cm^{-1}. [15] The absorbances were measured by keeping the concentration of the complex constant (1×10^{-4} M) while varying the DNA concentrations (1×10^{-4} M, 5, 10, 15, 20 and 25 µL).

Viscosity measurements: Viscosity titration experiments were carried on an Ostwald's viscometer at room temperature by varying the complex concentration at constant CT-DNA concentration (50 µM). Flow time of each sample was measured three times to calculate the average flow time using digital stop watch. Each sample was measured at least three times and average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ vs binding ratio ([complex]/ [DNA]), where η was the viscosity value for DNA in presence of the Cr (III) complexes and η_0 was the viscosity value of CT-DNA alone.

DNA cleavage: The cleavage of plasmid DNA was monitored using agarose gel electrophoresis. Supercoiled pUC19 (0.5 µg) in Tris-HCl buffer (50 mM) with 50 mM NaCl (pH 7.2) was treated with metal complexes (10^{-3} M). The samples were incubated for 1 h at 37 °C. A loading buffer containing 25 % bromophenol blue, 0.25 % xylene cyanol and 25 % glycerol were added and electrophoresis was performed at 70 V for 2 h in TBE buffer using 1.0 % agarose gel containing 1.0 µg/mL ethidium bromide. Bands were visualized using UV light and photographed. The cleavage efficiency was measured by determining the ability of the complex to convert the supercoiled DNA (Form I) to nicked circular form (Form II) or linear form (Form III).

Results and Discussion

The Schiff base ligand was obtained by the 1:1 condensation of 3-amino-2-methyl-4(*3H*)-quinazolinone with 2-pyridine carboxaldehyde. The formation of the Cr(III) complexes was achieved by reaction of the ligand with Cr (III) salts in 1:2 [M: L] and 1:1:1 ratio. The analytical and physical data are presented in Table 1.

IR spectra of Cr (III) complexes: The characteristic absorption in the IR spectra of complexes is listed in Table 2. Comparison of the infrared spectral data of complexes and ligand confirmed that complexation has occurred as significant shifts in the bands of the azomethine group $\nu_{CH=N}$ and lactum $\nu_{C=O}$ groups were observed. The expected mode of interaction between the Schiff base ligand and the Cr (III) ion was via coordination of the Cr(III) ion to the azomethine nitrogen group and lactum oxygen. The IR spectra of Cr(III) complexes showed the expected characteristic imine band in the region 1587-1582 cm^{-1}, shifted to lower frequiences due to metal coordination. A sharp band at 1684 cm^{-1} in the ligand due to $\nu_{C=O}$ was also shifted to lower frequency in the complexes. Moreover, the appearance of additional weak bands in the region 474-467 and 565-537 cm^{-1} which were attributed to ν(M-O) and ν(M-N), respectively, confirmed complexation .

Conductivity measurements: The molar conductance values of the Cr (III) complexes in DMF (10^{-3} M solutions) were measured at room temperature and the results are listed in Table 1. The conductance values of Cr (III) complexes fall in the range 14.5-12 $Ohm^{-1}cm^2mol^{-1}$, indicating the non-electrolytic nature of complexes.

Magnetic susceptibility: The room temperature solid state magnetic moment data of the complexes are reported in Table 1. The effective magnetic moment values (μ_{eff}) of Cr(III) complexes were calculated using the formula $\mu_{eff} = 2.84\ (\chi_{corr} \times T)^{1/2}$ B.M. The effective magnetic moments obtained for the complexes are in the range of 3.78-3.75 B.M. which is consistent with the presence of one unpaired electron.

Electronic spectra: The electronic absorption spectra of the Schiff base metal complexes in DMF were recorded at room temperature. Both the complexes 1 and 2 exhibit an absorption band in the range 360-390 nm, which are

assigned to charge transfer transition from the pπ-orbitals of the donor atoms to the d-orbitals of the metal. In addition, complexes exhibit d-d transition in the 617-724 nm range.

Thermal studies: The TG/DTG curves of complex 2 are illustrated in Figure 2. The TGA profiles over the temperature range 30-200 °C are usually due to loss of water of moisture, hydration and coordination. The first stage between 150 and 200 °C corresponds to the dehydration. The anhydrous complex is stable up to 200 °C. The observed weight loss indicates the loss of two coordinate water molecules present in the complex. In the second stage, continuous mass loss occurs in the range 200-320 °C (TG=42.1 %), suggesting the evaporation of ligand. The third stage between 320-530 °C, (TG=31.5 %) corresponds to the remaining organic ligand molecule leaving behind metal oxide as the end product.
Based on the above spectral studies, following structures are assigned for the complexes (Figure 3).

Biology activity

Antimicrobial activity: The data pertaining to the antimicrobial potential of ligand and its Cr(III) complexes are presented in Table 3. The ligand has poor activity against both bacteria and fungi. This activity may be due to the presence of imine group which imparts in elucidating the mechanism of transformation reaction in biological systems. The results indicate that the complexes show more activity than the ligand against same microorganisms under identical experimental conditions. This would suggest that the chelation could facilitate the ability of a complex to cross a cell membrane and can be explained by Tweedy's chelation theory [16]. All the test compounds show lesser activity than the standard antibiotics.

DNA binding studies: The interaction between the complexes and CT-DNA were evaluated by UV-Vis spectroscopy titration. The absorption spectra of complexes (1 and 2) in the presence of CT-DNA are shown in Figure 3. From the Figure 3, it is clear that, in both spectra of complexes 1 and 2, hyperchromicity is observed on the addition of CT-DNA. This significant hyperchromism effect suggests that there exists a strong interaction between the Cr(III) complex and DNA that can be rationalized in terms of intercalative binding mode [17].

Viscosity measurements: To confirm the interaction between the two complexes and DNA, viscosity measurements were carried out. A classical intercalation model results in lengthening of the DNA helix as base pairs to an increase of DNA viscosity[18]. The viscosity of CT-DNA is found to be increased in the presence of complexes. The extent of the viscosity increases caused by them is comparable, which is greater than that observed for ligand measured under the same conditions (Figure 5). This observation suggests that the mode of DNA binding by complexes involved base pair intercalation.

Nuclease activity: The nuclease activity of complexes 1 and 2 has been assessed by their ability to convert supercoiled pUC19 DNA from Form I to Form II and Form III by gel electrophoresis. Figure 6 shows the cleavage pattern of plasmid DNA. The DNA cleavage efficiency of the complexes was due to the different binding affinity of the complex to DNA. Both the complexes are able to cleave DNA to almost same extent in absence and presence of H_2O_2.

References

1. Karthikeyan M. S., Prasad D. J., Poojary B., Bhat K. S., Hollaa B. S. and Kumari N. S., *Bioorg. Med. Chem*., **14,** 7482-7489 (**2006**).
2. Holla B. S., Veerendra B., Shivananda M. K., Poojary B., *Eur. J. Med. Chem*., **38,** 759-76 (**2003**).
3. Raman N., Ravichandran S., Thangaraja C., *J. Chem. Sci*., **116,** 215-219 (**2004**).
4. Liu T., Lv J., Cai S., Wang X., Liu L., Wang Y., *J. Inorg. Biochem.*, **100,** 1888-1896 (**2006**).
5. Nair R., Shah A., Baluja S., Chanda S., *J. Serb. Chem. Soc*., **71,** 733-744 (**2006**).
6. Singh K., Barwa M. S., Tyagi P., *Eur. J. Med. Chem.*, **42,** 394-402 (**2007**).
7. Dhar S., Nethaji M., Chakravarty A. R., *Inorg. Chem.*, **45,** 11043-11050 (**2006**).
8. Dhara K., Ratha J., Manassero M., Wang X. Y., Gao S., Banerjee P., *J. Inorg. Biochem.*, **101,** 95-103 (**2007**).
9. Yang X.B., Wang L., Zhang J., Zhang Z. W., Lin H. H., Zhou L. H., Yu X. Q., *J. Enzym. Inhib. Med. Chem.*, **24,** 125-130 (**2009**).
10. Vleck Jr., *Coord.Chem.Rev*., **230,** 225-242 (**2002**).
11. Garcia-Friaza G., Fernandez-Botello A., Perez M., Prieto J., Moreno V., *J. Inorg. Biochem.*, **100,** 1368-1377 (**2006**).
12. Reichman M. E., Rice S.A., Thomas C.A., Doty P., *J. Am. Chem. Soc.*, **76,** 3047-3053 (**1954**).
13. Tweedy B. G., *Phytopathology.*, **55,** 910-914 (**1964**).
14. Vijayalakshmi R., Kanthimathi M., Subramanian V., Nair B.U., *Biochem, Biophys. Acta*., **1475,**157-164 (**2000**).
15. Satyanarayana S., Dabrowiak J. C., Chaires J. B., *Biochemistry.*, **31,** 9319-9324 (**1992**).
16. Liu C., Wang M., Zhang T., Sun H., *Coord.Chem.Rev.*, **248,** 147-168 (**2004**).
17. Ahmed I.T., *Spectrochim.Acta Part A.*, **65**, 5-10 (**2006**).
18. Lippert B., *Coord.Chem.Rev.*, **200,** 487-516 (**2000**).

Table 1: Analytical and physical data of the Cr (III) complexes

Compound	Molecular formula	Yield (%)	Calcd. (found), %				μ_{eff} B.M.	Molar conductance $\Omega^{-1}cm^2mol^{-1}$
			C	H	N	M		
1	$C_{36}H_{30}N_6O_6ClCr$	66	59.25 (59.49)	4.12 (4.17)	11.50 (11.91)	7.10 (7.27)	3.78	12.5
2	$C_{30}H_{25}N_5O_4ClCr$	61	59.40 (59.57)	4.12 (4.26)	11.55 (11.79)	8.58 (8.82)	3.75	14.2

Table 2: Important IR spectral bands of Cr (III) complexes

Compound	ν(C=N)	ν(C=O)	ν(M-O)	ν(M-N)
L	1597	1684	---	---
1	1587	1642	467	565
2	1582	1648	474	537

Table 3: Antimicrobial activity of Schiff base and its Cr (III) complexes

Compound	Zone of inhibition (in mm)*			
	Antibacterial activity		Antifungal activity	
	S.aureus	*E.coli*	*A.niger*	*F.oxysporum*
L	07	09	03	07
1	18	19	16	23
2	21	23	19	27
Chloramphenicol	32	29	-	-
Griseofulvin	-	-	27	36

*average of three replicates

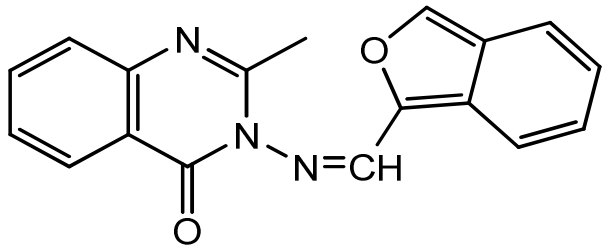


Figure 1: Structure of 3-(isobenzofuran-1-ylmethyleneamino)-2-methylquinazolin-4(*3H*)-one (L)

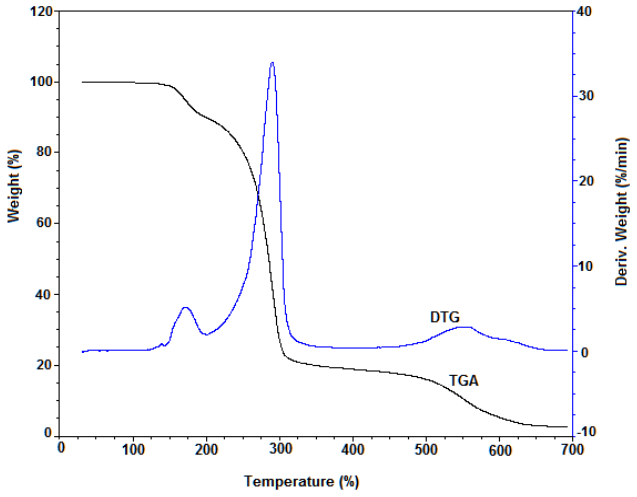
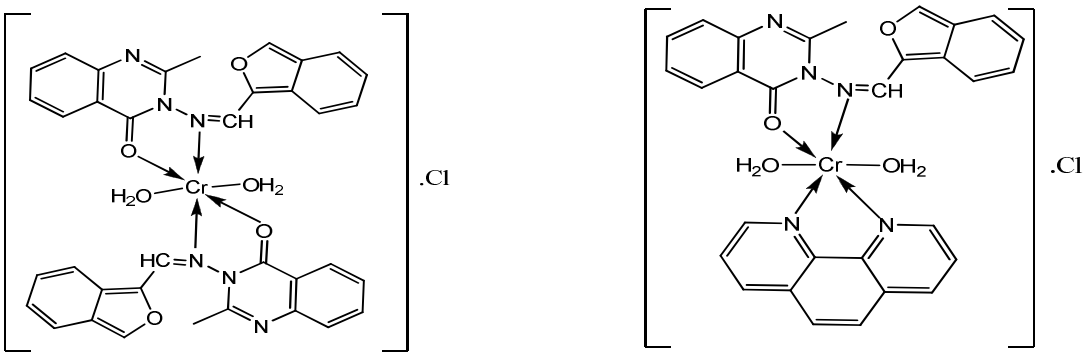
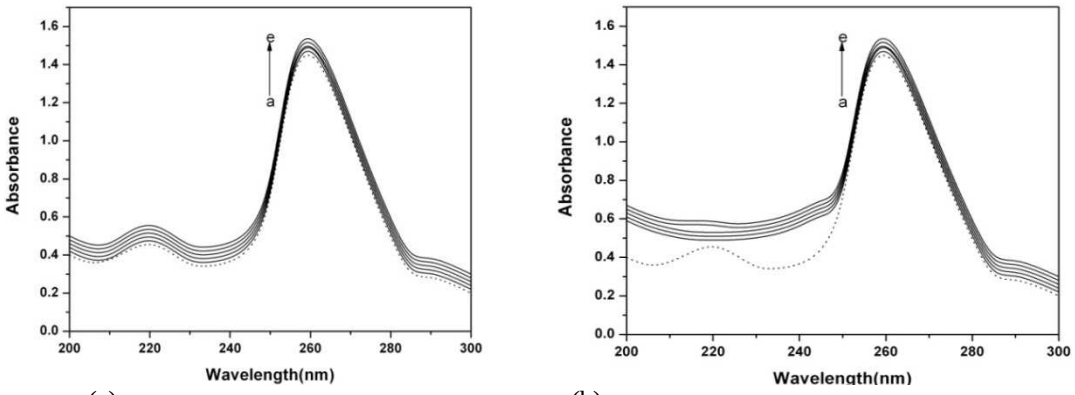


Figure 2: Thermogravimetric (TGA and DTG) curves of complex 2



1 **2**

Figure 3: Structures of chromium (III) complexes



(a) (b)

Figure 4: Absorption spectra of (a) 1 and (b) 2, in Tris-HCl buffer upon addition of DNA = 1×10^{-4} M, 0-25 µL. Arrow shows the absorbance changing upon increasing the concentration of DNA. (---) indicates absorption of DNA alone

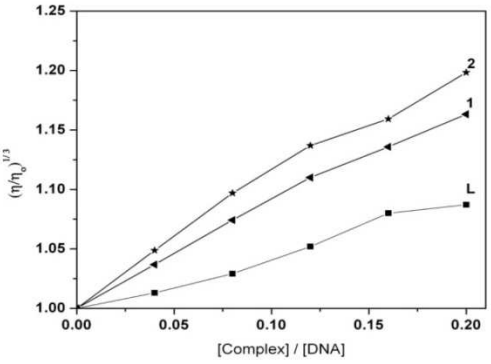


Figure 5: Effect of increasing amounts of complexes and ligand on the relative viscosity of CT-DNA

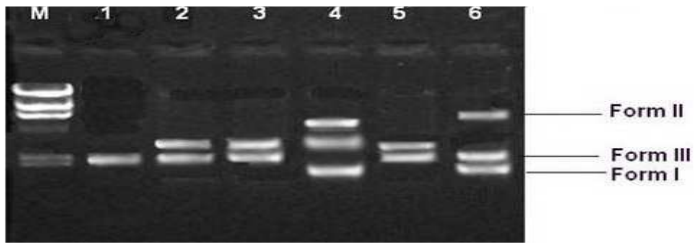


Figure 6: Cleavage of supercoiled pUC19 DNA (0.5 µg) by complexes 1 and 2 in a buffer containing 50 mM Tris-HCl at 37 °C (30 min): lane M: marker, lane 1: DNA control, lane 2: DNA+H_2O_2, lane 3: complex1+ DNA, lane 4: complex 1 (10^{-3} M)+DNA + H_2O_2, lane 5: complex 2 (10^{-3} M) + DNA, lane 6: complex 2 + DNA+H_2O_2