Research Article

Zinc (II) Complexes as Antimicrobial and DNA Cleaving agents: Synthesis and Spectral Characterization

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ABSTRACT

The Zinc (II) complexes of 4(3*H*)-quinazolinone derived Schiff base ligand were prepared. Both free ligand and its Zinc (II) complexes were characterized by elemental analyses, spectral methods (IR, ^{1}H-NMR, MS and UV–Vis), molar conductance measurements and thermal studies. The bidentate ligand coordinated to the Zinc (II) 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. The nuclease activity was performed using supercoiled pUC19 plasmid DNA.

Keywords: Schiff base, Zinc (II) complexes, spectral studies, antimicrobial activity, DNA cleavage.

INTRODUCTION

Schiff base of 4(3*H*)-quinazolinone and its complexes have a variety of applications in physiological, clinical, analytical and pharmacological areas[1,2]. Studies of a new kind of chemotherapeutic Schiff bases are now attracting the attention of biochemists[3]. Deoxyribonucleic acid (DNA) is the primary target molecule for most anticancer and antiviral therapies according to cell biologists. Investigations on the interaction of DNA with small molecules are important in the design of new types of pharmaceutical molecules. A number of metal chelates, as agents for mediation of strand scission of duplex DNA and as hemotherapeutic agents, have been used as probes of DNA structure in solution[4] Spectroscopic and voltammetric studies of copper (II) complexes of bis(pyrid-2-yl)-di/trithia ligands bound to calf thymus DNA[5]. Due to relatively low bioavailability of $ZnCl_2$, the coordination Chemistry of zinc (II) ion was explored. The first orally active insulin-mimetic and antidiabetic zinc(II)–picolinate complexes were discovered in 2002[6]. Metallokinetic Study of Zinc in the Blood of normal rats gave Insuline mimetic Zinc(II) complexes and improvement of Diabetes Mellitus in Type 2 Diabetic GK rats by their Oral Administration. Later wide variety of zinc(II) complexes with different coordination structures have been synthesized[7].

In this paper we report the synthesis, characterization, redox, antimicrobial and DNA cleavage studies of Zinc(II) complexes with a Schiff base ligand derived from 3-amino-2-methyl-4(*3H*)-quinazolinone and 2-benzofuran carboxaldehyde.

EXPERIMENTAL

Materials and Methods

Chemicals used: 3-amino-2-methyl-4(*3H*)-quinazolinone and 2-benzofuran carboxaldehyde was obtained from Aldrich. Zinc(II) salts and solvents were commercially available of high purity. pUC19 DNA from *GENEI* 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. The UV-Visible 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 Bruker-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)

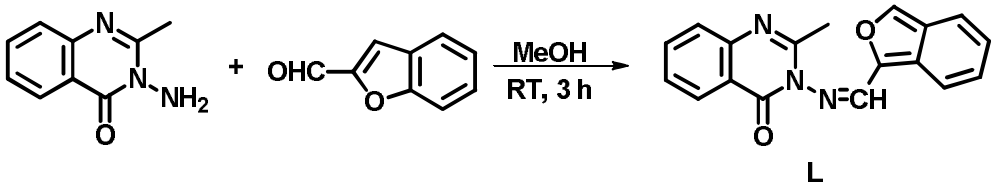
A 1:1 equimolar solution of 3-amino-2-methyl-4(*3H*)-quinazolinone (0.350 g, 2 mm. mol) and 2-benzofuran carboxaldehyde (0.263 g, 2 mm. mol) were mixed in 30 mL methanol and gently heated for 3 h with constant stirring. The characteristic yellow precipitate of Schiff base obtained by condensation was 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 Zinc(II) complexes of Schiff base were prepared in 1:2 [metal:ligand] and 1:1:1 [metal:ligand:1,10-phenanthroline] stoichiometric proportions (Figure 1).

To a 20 ml hot methanolic solution of metal chloride (0.170 g, 1 mmol), 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 reaction mixture was refluxed for 6 h at 60 °C. The solid complex precipitated was filtered, washed thoroughly with ethanol and dried *in vacuo*. The analytical and physical data were reported in Table 1.



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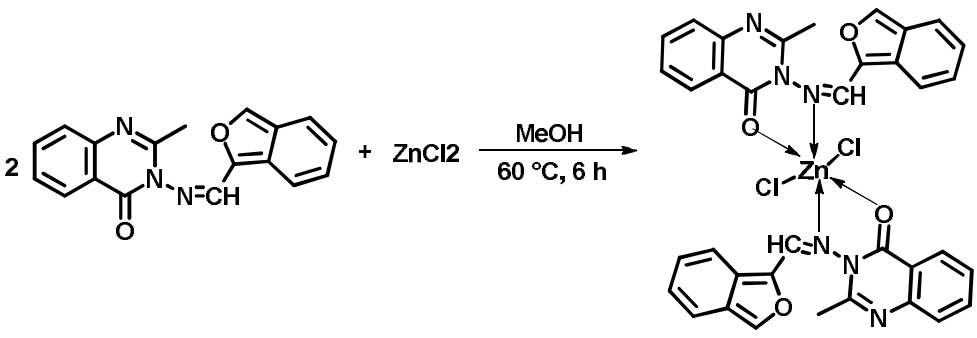

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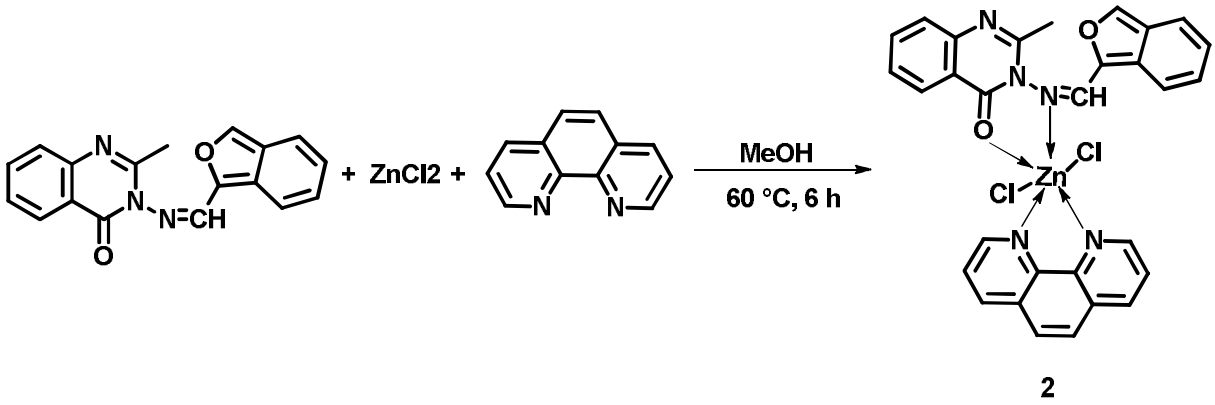


Fig. 1: synthetic route of 3-(isobenzofuran-1-ylmethyleneamino)-2-methylquinazolin-4(*3H*)-one (L) and its Zinc(II) complexes

Biological activity

Antimicrobial studies

In the present study, the antimicrobial activity of the ligand (**L**) and its Zinc(II) complexes were evaluated against one Gram-positive (*R.solanacearum*), two Gram-negative bacteria (*B.subtilis* and *S.aureus*) and two fungi (*A.niger* and *A.solani*) by paper disc diffusion method[8]. 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 DMF (no inhibition activity) to get concentration of 1 mg/mL. The bacteria were sub-cultured in agar medium, and the Petri dishes were incubated for 24 h at 37 °C. Standard antibacterial drug (Chloramphenicol) was also screened under similar conditions for comparison. The fungi were sub-cultured in potato dextrose agar medium and the Petri dishes were incubated for 72 hours at 37 °C. Standard antifungal drug (Griseofulvin) was used for comparison. The wells were dug into the agar media using a sterile metallic borer. Activity was determined by measuring the diameter of the zone showing complete inhibition (mm).

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[9]. Bands were visualized using UV light and photographed. The cleavage efficiency was measured by determining the ability of the complex to convert the super coiled DNA (Form I) to nicked circular form (Form II) or linear form (Form III).

RESULT AND DISCUSSION

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

Table 1: Analytical and physical data of the Zinc (II) complexes

Compound	Molecular formula	Yield (%)	Calcd. (found), %				Molar conductance Scm^2mol^{-1}
			C	H	N	M	
1	$ZnC_{36}H_{30}N_6O_6Cl_2$	69	60.17 (60.79)	4.12 (4.17)	11.50 (11.91)	7.10 (7.27)	12.58
2	$ZnC_{30}H_{25}N_5O_4Cl_2$	67	59.81 (60.13)	4.12 (4.26)	11.55 (11.79)	8.58 (8.82)	13.26

IR spectra of Zinc (II) Complexes

The IR spectra of complexes are listed in Table 2. The significant shift on the bands of the azomethine group $v_{C=N}$ and lactum $v_{C=O}$ groups of the ligand confirmed the complexation. The expected mode of interaction between the ligand and the Zinc(II) ion was *via* coordination of the Zinc(II) ion to the azomethine nitrogen group and lactum oxygen[10]. The IR spectra of Zinc (II) complexes showed the expected characteristic imine band in the region 1587-1582 cm^{-1}, shifted to lower frequencies due to metal coordination. A sharp band at 1684 cm^{-1} in the ligand due to $v_{C=O}$ was also shifted to lower frequency in the complexes. The appearance of additional weak bands in the region 474-467 and 565-537 cm^{-1} due to v(M-O) and v(M-N) respectively, confirmed complexation.

Table 2: Important IR spectral bands of Zinc (II) complexes

Compound	v(C=N)	v(C=O)	v(M-O)	v(M-N)
L	1597	1684	---	---
1	1584	1669	453	529
2	1579	1673	467	533

Conductivity Measurements

The molar conductance values of the Zinc(II) complexes in DMF (10^{-3} M solutions) were measured at room temperature and the results are listed in Table 1. The conductance values of Zinc(II) complexes fall in the range 14.0-12.0 Scm^2mol^{-1}, indicating the non-electrolytic nature of complexes.

Electronic Spectra

The electronic absorption spectra of the Schiff base metal complexes in DMF were recorded at room temperature. The ligand showed the absorption bands at 318 and 303 nm, which are assigned to π-π* transition of the C=N chromophore. Upon complexation, this band was shifted to lower wavelength region, suggesting the coordination of azomethine nitrogen with Zinc(II) ion. Zinc(II) complex does not exhibit d-d electronic transition due to the non-availability of d-electrons. An absorption band was observed in the range 21.0-22.50 kK due to M→L CT transitions which confirms its tetrahedral geometry[11].

Thermal Studies

The TG/DTG curves of complex 1 are illustrated in Figure 2. The decomposition of the complex in 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 the ligand. The third stage between 320-530 °C, (TG=31.5 %) corresponds to the remaining organic molecule of the ligand leaving behind metal oxide as the end product[12].

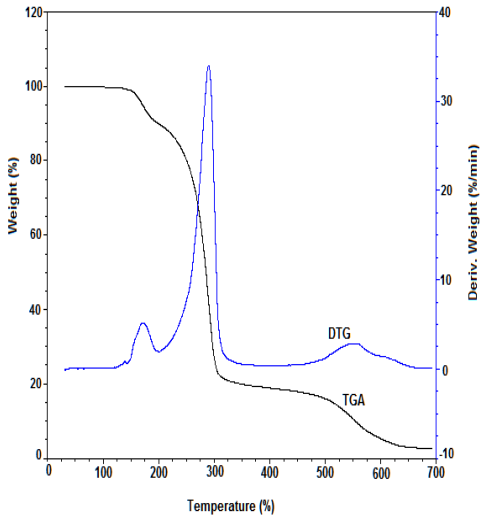
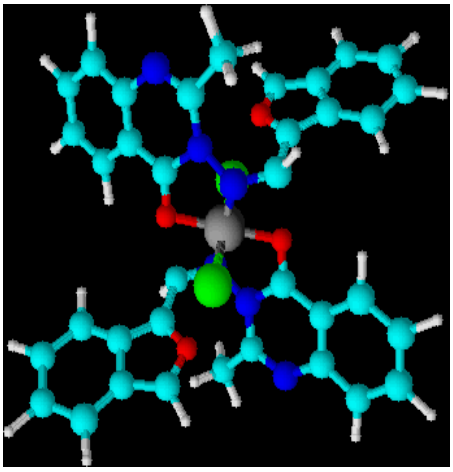


Fig. 2: Thermo gravimetric (TGA and DTG) curves of complex 2



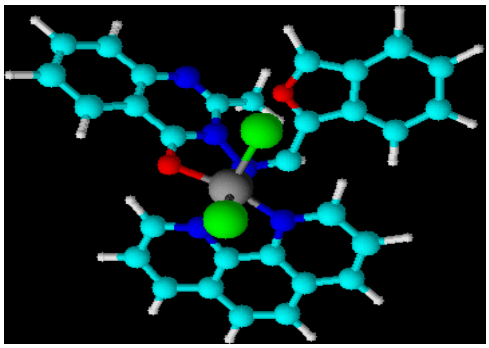


Fig. 3: Optimized structures of Zinc (II) complexes

Antimicrobial Activity

Table 3 indicates the antimicrobial potential of ligand and its Zinc (II) complexes. By the observation of zones of inhibition, it was concluded that the organotin complexes are more active than free ligands, which indicates that the metallation increases antimicrobial activity. The above studies reveal that the Zinc(II) complexes synthesized in the present work are highly active against all the selected microorganisms. The results reported in Table 3 reveal that all the Zinc(II) complexes are particularly active against bacteria *S.aureus* and against fungus namely, *A.solani.* The complexes showed moderate activity against other selected species of microorganisms. 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[13]. All the test compounds show lesser activity than the standard antibiotics.

Table 3: Antimicrobial activity of Schiff base and its Zinc (II) complexes

Compound	Zone of inhibition (in mm)*				
	Antibacterial activity			Antifungal activity	
	B.subtilis	*S.aureus*	*R.solanacearum*	*A.niger*	*A. solani*
L	07	09	05	03	07
1	18	19	18	16	30
2	21	23	20	19	31
Chloramphenicol	32	29	26	-	-
Griseofulvin	-	-	-	27	36

*average of three replicates

Nuclease Activity

The nuclease activity of complexes 1 and 2 has been assessed by their ability to convert super coiled pUC19 DNA from Form I to Form II and Form III by gel electrophoresis. Figure 4 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.

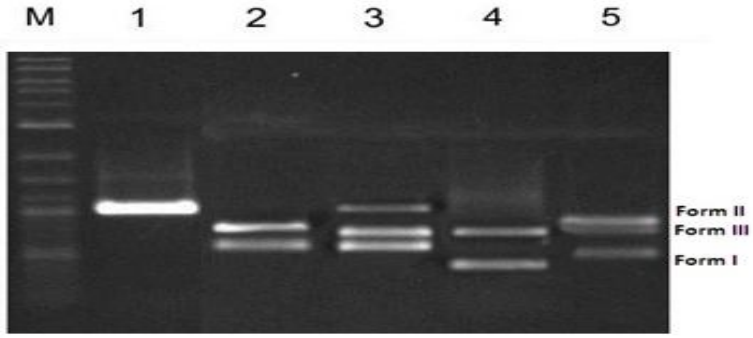


Fig. 4: Cleavage of supercoiled pUC19 DNA (0.5 µg) by the Zinc(II) complexes 1 and 2 in a buffer containing 50 mM Tris-HCl at 37 °C (30 min): lane M: marker; lane 1:DNA control; lane 2: complex 1 (10^{-3} M) + DNA; lane 3: complex 1 (10^{-3} M) + DNA + H_2O_2; lane 4: complex 2 (10^{-3} M) + DNA; lane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

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