

RESEARCH ARTICLE

SYNTHESIS, CHARACTERISATION, THERMAL ANALYSIS AND DNA CLEAVAGE ACTIVITY OF A NOVEL ZINC (II) COMPLEX OF PYRAZOLE SCHIFF BASES

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ABSTRACT

A hydrazone Schiff base Zn(II) metal complex is synthesised from the Schiff base ligand Thiophene-2-carboxylic acid hydrazide and 1, 3-diphenyl-1*H*-pyrazole-4-carboxaldehyde reacted together in 1:1 mole ratio to obtain Schiff base ligand (**HL**) which was subsequently, allowed to react with Zn(CH₃COO)₂·2H₂O. The Schiff base ligand and its Zn (II) complex prepared were characterized on the basis of elemental analysis, thermogravimetry, UV-Visible spectroscopy, FT-IR spectroscopy and NMR spectroscopy. IR spectrum of the zinc complex shows that the ligand (**HL**) is coordinated to the metal ion in monoanionic bidentate fashion with the 1:2 metal to ligand stoichiometry. The thermal behaviour of the complex shows a single step decomposition pattern leaving the respective ZnO residue. The DNA cleavage activity of the complex is monitored using agarose gel electrophoresis method which indicates the potential of the complex to cleave supercoiled DNA.

Keywords: Characterisation, Thermal analysis, DNA Cleavage.

1. INTRODUCTION

Schiff base Hydrazone are of significant interest and attention because of their biological activity including anti-tumour, anti-bacterial, anti-fungal and anti-carcinogenic properties and catalytic activity (1). Schiff bases readily coordinate with metal ions with various modes of coordination under different reaction conditions and their metal complexes have potent chemical, physical, biological and catalytic properties (2,3,4). Schiff bases of pyrazole heterocycles found their place in different fields of chemistry because of their wide biological activity like antimicrobial (5), anti-inflammatory (6), anti tubercular (7), anti tumor (8), anti angiogenesis (9), anti parasitic (10), anti viral (11) and also possesses analgesic and anxiolytic activity (12). Many transition metal pyrazole Schiff base complexes are reported to have biological importance (13). Copper pyrazole complexes were found to be effective apoptosis inducers and inhibited angiogenesis on Matrigel and HUVEC migration *in vitro* (14). Some palladium pyrazole Schiff base complexes were synthesized and characterised for their cytotoxic effect against the fast growing head and neck squamous carcinoma cells SQ20B and SCC-25 and were found to have higher clonogenic cytotoxic effect than cisplatin when tested on SQ20B cell line (15). Among the transition metal complexes, zinc complex have a significant biological importance due to their presence in various enzymes and proteins (16). In this work, we synthesised zinc complex of pyrazole heterocyclic and characterised by elemental analysis, FT-IR, UV visible and NMR

techniques. From the data obtained, a 1:2 metal to ligand tetrahedral coordination geometry was proposed for Zn complex.

2. MATERIALS AND METHODS

Reagent grade chemicals were procured commercially and used without subsequent purification. 1,3-diphenyl-1*H*-pyrazole-4-carboxaldehyde and thiophene carboxylic acid hydrazide were purchased from Sigma Aldrich. [Zn(Ac)₂·2H₂O] were purchased from Lobachem and Rankem. The commercial solvents were used without further purification.

2.1. Physical measurements

Melting Points of the samples were determined using Raaga apparatus. FT-IR spectra of solid sample of ligands and complex were recorded using KBr pellets on a Nicolet Avatar instrument in the frequency range 400-4000 cm⁻¹. Microanalyses (C, H & N) were performed on a Vario EL III CHNS analyser. Electronic absorption spectra of the samples were recorded using a Jasco V-630 spectrophotometer. ¹H NMR spectrum of the ligand was recorded on a Bruker Avance-3 spectrometer at 400 MHz.

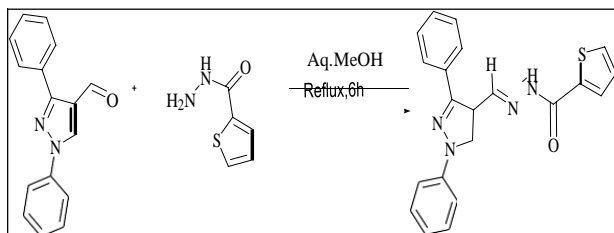
2.2. Synthesis of schiff base

The Schiff base was prepared by reacting a mixture of thiophene carboxylic acid hydrazide (0.273 g, 1 mM) and 1,3-diphenyl-1*H*-pyrazole-4-carboxaldehyde (0.173g, 1mM) in 50 mL of aqueous methanol. A few drops of glacial acetic acid were added to the reaction mixture. The resulting

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solution was refluxed for 6 h, cooled and the precipitate obtained was checked for purity. The analysis of the product by TLC revealed the formation of the ligand. Synthetic scheme for the preparation of the Schiff base is given in below.

Yield: 85%; Colour: Pale yellow; Melting Point: 210°C.

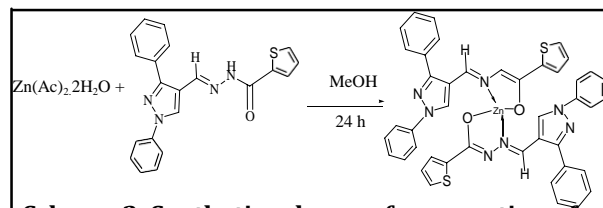


Scheme 1 Synthetic scheme for the preparation of Schiff base

2.3. Synthesis of complex

Methanolic solution of $[Zn(Ac)_2 \cdot 2H_2O]$ (0.1455 g; 0.5 mM) was refluxed with equimolar quantity of the ligand, (0.217 g; 0.5 mM) in 20 mL of methanol for 24 h (scheme 2). The solution was kept for evaporation to yield the yellowish white colour complex which was washed several times with petroleum ether and the purity of the complex was checked by TLC. The trials to crystallize the complex failed miserably.

Yield: 43 %; Colour: White; Melting point: 215 °C.



Scheme 2. Synthetic scheme of preparation of zinc complex

2.4. Determination of oxidative plasmid DNA strand breakage

Generally DNA damage is indicated by the conversion of supercoiled form of plasmid DNA to circular form. The potential of newly synthesized complex to cause oxidative plasmid DNA breakage was assessed by the plasmid DNA breakage assay (17). The 20, 30 and 40 μ M concentration of the test compounds were added to 500 ng of pBR322 supercoiled plasmid DNA along with the blank and incubated for 6h at ambient temperature under dark. Then, the samples were mixed with 6X orange loading dye (Fermentas, Mumbai) and loaded into 1% agarose gel containing Ethidium bromide. After 30 min of gel run, the extent of damage caused by the test compounds were visualized under UV light

and documented using G-BOX (GE-health care, USA).

3. RESULTS AND DISCUSSION

The Schiff base ligand is synthesised by reacting equimolar quantities of thiophene carboxylic acid hydrazide and 1,3-diphenyl-1H-pyrazole-4-carboxaldehyde in methanol medium to yield pale yellow colour ligand thiophene 2-carboxylic acid (1,3 diphenyl 4,5- dihydro-1H-pyrazol-4-yl methylene)-hydrazide (HL). The reactions of the ligand with $[Zn(Ac)_2 \cdot 2H_2O]$ in

methanol medium yielded complex of composition $[Zn(L)_2]$ (Scheme 2). Analytical data of the Schiff base ligand and its zinc complex are given in Table.1 and are in well agreement with the proposed molecular formulae. Zinc complex is white in colour, non-hygroscopic solid and stable in air. It is sparingly soluble in common organic solvents and completely soluble in DMF and DMSO. The ligand and the complex are characterized using IR, UV-visible and NMR spectroscopic techniques and elemental analysis method. Thermal analysis of the Zinc complex was done to discover its formation as proposed.

3.1. Analytical data

Analytical data of the ligand (HL) and complex are given in table.1 and they are in good agreement with the expected values.

Table.1. Analytical data of ligand and complex

Molecular formula	Mol. Wt	C %		H %		N %	
		Fou nd	Calc .	Fou nd	Calc .	Fou nd	Calc .
$C_{21}H_{16}N_4O_2$	372.44	67.43	67.72	4.71	4.33	14.95	15.04
$C_{42}H_{30}ZnN_8O_2S_2$	807.26	62.05	62.41	3.03	3.74	13.69	13.86

3.2. FT-IR spectral data of the ligand and complex

IR spectrum of the ligand showed a sharp band in the region 3232 cm^{-1} due to the presence of $\nu_{(N-H)}$ stretching vibrations. A very strong band found around 1647 and 1598 cm^{-1} was assigned as due to amide carbonyl symmetric and asymmetric stretching vibration. The other bands at 1547 and 1073 cm^{-1} were assigned to the $\nu_{(C=N)}$ and $\nu_{(N-N)}$ stretching frequencies of the ligand.

The bands due to $\nu_{(C=O)}$ and $\nu_{(N-H)}$ stretching vibrations of the hydrazones were absent in the IR spectra of these complex with the appearance of two new bands between $1547-1493\text{ cm}^{-1}$ due to the

$\nu_{(C=N-N=C)}$ function generated with the enolisation followed by deprotonation and attested the coordination of hydrazone ligand in the enol form. Furthermore, a decrease in the $\nu_{(C=N)}$ stretching frequency involving the azomethine nitrogen indicated its involvement in the coordination to the metal ion [18].

The analytical data and IR characteristics are in good agreement with the proposed structure of Zinc complex. The important IR stretching frequencies of the ligand and complex are given in the Table 2. The IR spectrum of ligand and complex is shown in Figure 1 and 2.

Table.2. IR data of ligand and complex

Compound	ν (N-H)	ν (C=O)	ν (C=N)	ν (N-N)
HL	3282	1647	1598	1073
[Zn(HL) ₂]	3169	-	1548	1069

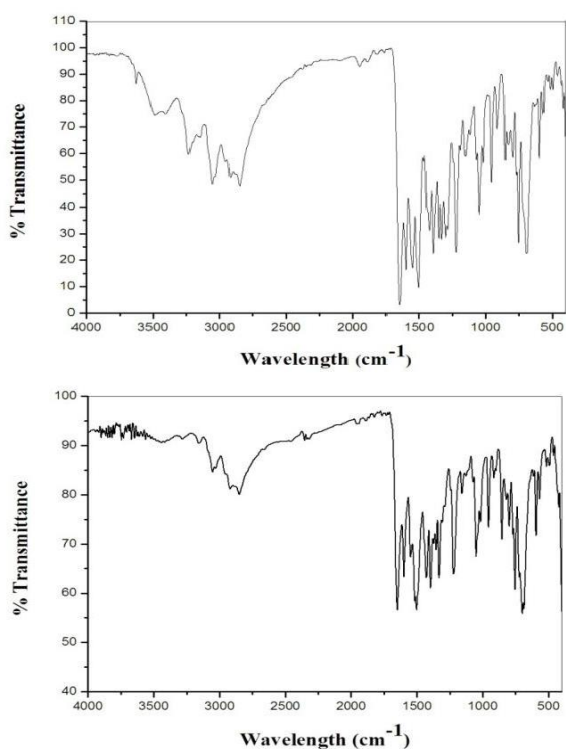


Fig.1. IR spectrum of ligand and zinc complex

3.3. ELECTRONIC SPECTRUM

The electronic spectra of the ligand and complex were recorded in DMSO solution. The ligand spectrum exhibited one broad band in the range 240-360 nm were assigned to the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ intra ligand transitions which were of higher energy transitions. The spectrum of complex exhibited two bands in the range 240-380 nm

region. The higher energy bands below 300 nm are attributable to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ intra ligand transitions [16]. Other broad band that was observed in the 300-370 nm regions can be assigned to a ligand to metal charge transfer (LMCT) transitions of the imine group.

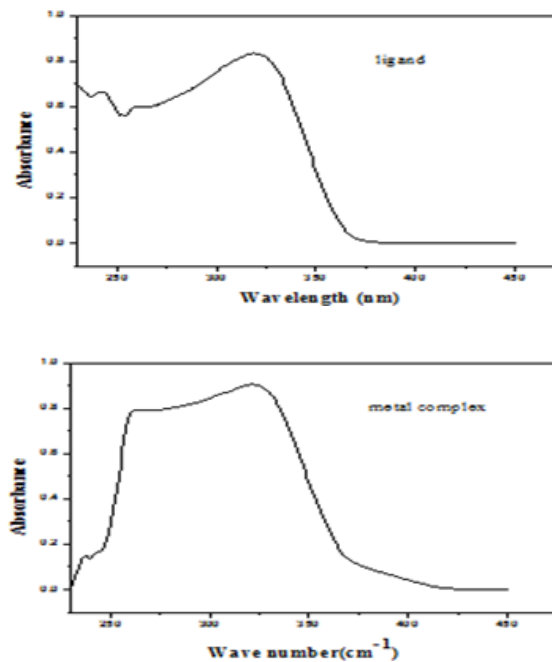
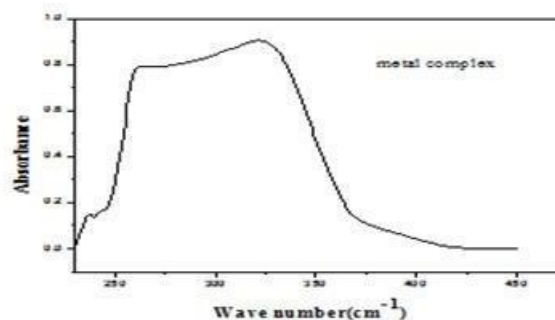


Fig.2. Electronic spectrum of ligand and Zinc complex

3.4. Proton NMR spectrum of the ligand

¹H-NMR spectrum of the free hydrazone ligand recorded using CDCl₃ as solvent was assigned on the basis of observed chemical shift. The spectrum displayed a singlet due to an NH proton in 9.0 ppm. The ligand showed a sharp singlet for azomethine (HC=N) at 8.87 ppm. Signals corresponding to the protons of benzene proton and thiophene proton of the ligand were observed as multiplets in the range of 7.22-8.58 ppm. NMR spectrum of the ligand ascertained its formation as expected.



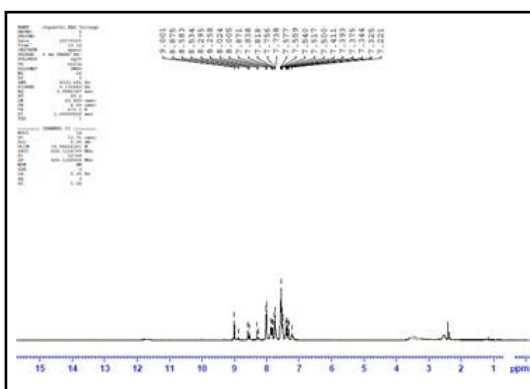


Fig. 3. ^1H NMR spectrum of the ligand

3.5. Thermal analysis of the complex

Thermo-gravimetric analysis of the zinc complex showed a single step decomposition pattern in the temperature range 180-356°C. The coordinated ligand present in the complex decomposed exothermically to yield ZnO residue. The percentage weight loss for the decomposition was found to be 89.97% for the decomposition of the two ligands. The remaining 10.03% matches with the residue ZnO. Thus it confirmed the formation of the complex as proposed in the Scheme 2.

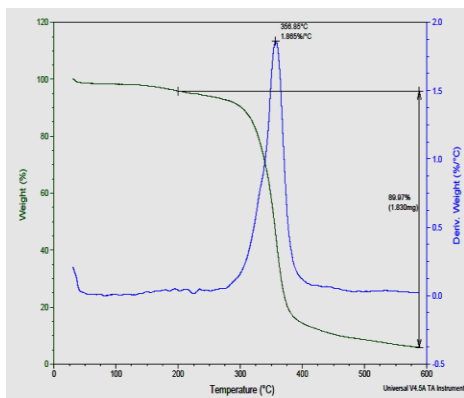


Fig. 8. TG-DTA curve of Zinc complex

Based on the above facts, a four coordinate tetrahedral geometry is proposed for the Zinc complex with 1:2 metal to ligand stoichiometry and the structure is given below.

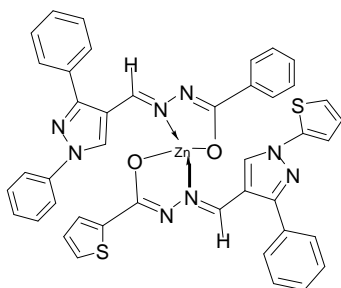


Fig.4. Proposed structure of zinc complex

3.6. DNA cleavage study

To check the role of synthesized complex on DNA breakage, plasmid DNA damage assay was performed, using the pBR322 plasmid DNA and the efficiency of the cleavage was monitored by agarose gel electrophoresis. The DNA cleavage efficiency of the complex was due to the difference in the binding affinity of the complex to DNA. Results of the experiment revealed that complex significantly damaged the plasmid DNA upon treatment for 30 min (Fig.5). The efficiency of studied complex to cleave super-coiled DNA to linear form is the characteristic of anticancer drugs those could effectively bind to the nuclear DNA and impart damage to it and thus arrest the proliferation of cancerous cells.

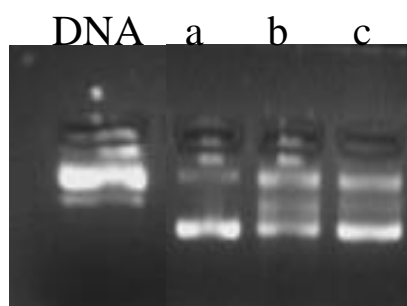


Fig. 5. pBR322 plasmid DNA cleavage by using different concentration of complex

4. CONCLUSION

Interesting coordination modes of hydrazone and their biological perspective provoked us to synthesize new zinc hydrazone complex by using the ligand prepared from 1,3 diphenyl pyrazole-1H 4carbaldehyde and thiophene carboxylic hydrazide (HL). The ligand was characterised by FT-IR, UV-visible and NMR spectral method showed its formation as expected. The elemental data of the ligand and the complex are in good agreement with the proposed molecular formulae of them. The IR spectral data of the zinc complex indicated the absence of N-H and carbonyl stretching vibrations and formation of new C=N vibrations. This shows the coordination of the ligand in monoanionic bidentate fashion in complex. UV spectral data of the complex showed four bands, two due to ligand centred transitions at 240-310 nm and another two metal centred transition in the range 320-450 nm. Based on the above spectral data tetrahedral geometry was proposed with 1:2 co-ordination of metal to ligand in which ligand formed NO chelate with the metal centre. The DNA cleavage studies showed that the complex have the potential to cleave DNA.

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