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**RESEARCH ARTICLE**

# SYNTHESIS, CHARACTERISATION, THERMAL ANALYSIS AND DNA CLEAVAGE ACTIVITY OF COPPER PYRAZOLE SCHIFF BASE

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## ABSTRACT

Thiophene-2-carboxylic acid hydrazide and 1, 3-diphenyl-1*H*-pyrazole-4-carboxaldehyde reacted together in 1:1 mole ratio to form Schiff base ligand(**L**) which was subsequently, reacted with $CuCl_2.2H_2O$formed complex of the composition ($Cu(L)Cl_2$). The Schiff base ligand and its Cu(II) complex were characterized on the basis of elemental analysis,thermogravimetry, UV-Visible spectroscopy, FT-IR spectroscopy and NMR spectroscopy. IR spectrum of the copper complex shows that the ligand coordinated through imine nitrogen and amide oxygen atom forming a neutral bidentate chelate with the metal centre.The thermal behaviour of thecomplex shows a single step decomposition pattern leaving CuO residues.DNA cleavage activity of the complexshowed the potential of the complex to cleave supercoiled DNA.

**Keywords:** Characterisation, Thermal analysis , DNA Cleavage.

## 1. INTRODUCTION

Schiff bases, theimportant class of ligands can readily coordinate with different metal ions forming stable chelate complexes. They are of synthetic importance due to the various modes of coordination under different conditions and exhibiteddifferent chemical, physical, biological and catalytic properties (1-3). Schiff bases of pyrazole heterocycles found their place in different fields of chemistry because of their wide biological activity like antimicrobial (4), anti-inflammatory (5), antitubercular (6), antitumor (7), antiangiogensis (8), antiparasitic (9), antiviral (10) and also possessesanalgesic and anxiolytic activity (11). Many transition metal pyrazole Schiff base complexes have been synthesized and tested for their biological activity (12). Copper (II) complexes show distorted octahedral and tetrahedral symmetries due to $d^9$configuration (Jahn Teller effect). The distortion is usually seen as axial elongation consistent with the lability and geometric flexibility of the complex.The fundamental role of copper and the recognition of its complexes asbioactive compounds created interest in their synthesis and their potential application inpharmaceutical industry. Copper complexes are of particular interest with regard to DNA cleavage through oxidative pathways (13).

Some pyrazole Schiff base complexes showed better 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 (14). Copper complexes have a significant place due to its presence in various enzymes and proteins. Copper pyrazole complexes were found to be one of the most effective apoptosis inducers and inhibited angiogenesis on Matrigel and HUVEC migration *in vitro* (15). Thus, in the present work, synthesis of copper complex of pyrazole heterocycle and its characterisation by elemental analysis, FT-IR, UV visible and NMR techniques.

## 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. $CuCl_2$. $2H_2O$ purchased from Lobachemie Pvt. Ltd. and Rankem. The commercial solvents were used.

### *2.1. Physical measurements*

Melting points of the sample were determined using Raaga apparatus. FT-IR spectra of solid sample of ligand and the 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 ligand (L)*

The Schiff base ligand was prepared by reacting a mixture of thiophene carboxylic acid hydrazide (0.273g, 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

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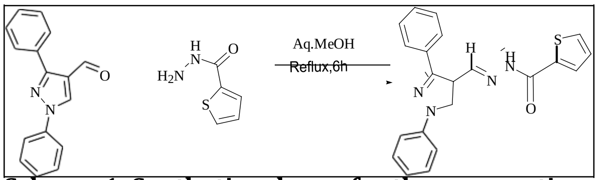
resulting solution was refluxed for 6 h, cooled and the precipitate obtained was checked for purity. The analysis of the product by TLC revealed the formation of the ligand. Synthetic scheme for the preparation of the Schiff base is given in below.

Molecular formula: $C_{21}H_{16}N_4OS$, Yield: 85%, Colour:Pale yellow, Melting Point: 210°C, Elemental analysis $C_{21}H_{16}N_4OS$, Found (Calc.) in %: C: 67.43(67.72), H:4.21(4.33), N: 14.95 (15.04)

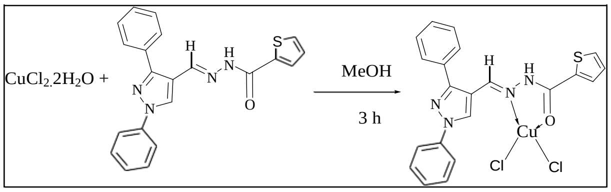


**Scheme 1. Synthetic scheme for the preparation of ligand**

*2.3. Synthesis of complex*

Methanolic solution of ($CuCl_2.2H_2O$) (0.162g; 0.5 mM) was refluxed with equimolar quantity of the ligand L (0.1627g; 0.5 mM) in 20 mL of methanol for 3 h (Scheme 2). After two hours, the reddish brown colour complex is precipitated which was filtered, washed several times with petroleum ether and water and then dried *in vacuum*. The purity of the complex was checked by TLC that showed single spot.

Molecular formula: $C_{21}H_{16}Cl_2CuN_4OS$, Yield: 44 %, Colour: reddish brown, Melting point: 230°C, Elemental analysis $C_{21}H_{16}Cl_2CuN_4OS$, Found (Calc.)in %: C: 49.68(49.76), H: 3.11(3.18), N: 10.67(11.05)



**Scheme 2. Synthetic scheme of preparation of copper complex**

*2.4. Determination of oxidative plasmid DNA strand breakage*

The potential of newly synthesized complex to cause oxidative plasmid DNA breakage was assessed by the plasmid DNA breakage assay followed by our previous protocol (16). The 10, 20and 30 µMconcentration 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 sample is mixed with 6X orange loading dye (Fermentas, Mumbai) and loaded into 1% agarose gel containing Ethidium bromide. After 30 minutes 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

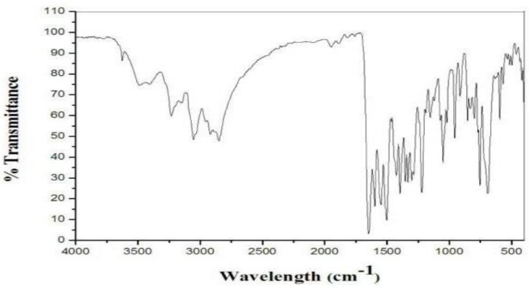
We synthesized the ligand by reacting equimolar quantities of thiophene carboxylic acid hydrazide and 1,3-diphenyl-1*H*-pyrazole-4-carboxaldehyde in methanol medium to yield pale yellow colour ligand thiophene 2-carboxylic acid (1,3 diphenyl 4,5- dihydro-1H- pyrazol-4-yl methylene)-hydrazide (L). The reactions of L with ($CuCl_2.2H_2O$) in methanol medium yielded complex of composition ($Cu(L)Cl_2$)(Scheme **2**). Analytical data of the Schiff base ligand and its copper complex are in well agreement with the proposed molecular formulae. The yellowish brown complex is non-hygroscopic solid and stable in air. It is sparingly soluble in common organic solvents, but soluble in DMF and DMSO. The ligand and the complex are characterized using IR, UV-visible, NMR spectroscopic techniques and elemental analysis. Thermal analysis of the complex was done to ascertain its formation as proposed.

*3.1. FT-IR spectral data of the ligand and complex*

FT-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 the $\nu_{(N-H)}$ and $\nu_{(C=0)}$ vibrations remained intact in the IR spectrum of thecomplex but were present at lower frequencies, implied the coordination of amide carbonyl oxygen and imine nitrogen with the copper centre (17). Thus the ligand coordinated to the metal as neutral bidentate fashion. The strong band appeared in the IR spectrum of the copper complex at 1582 $cm^{-1}$ is assigned to $\nu_{(C=N)}$ stretching frequency of the pyrazole ring.

The analytical data and IR characteristics are in good agreement with the proposed structure of copper complex.



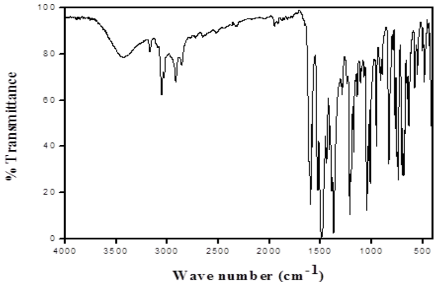


**Fig. 1. IR spectrum of ligand and complex**

*3.2. 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→π* and π→π* intra ligand 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→π* and π→π* intra ligand transitions (17). 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 (14).

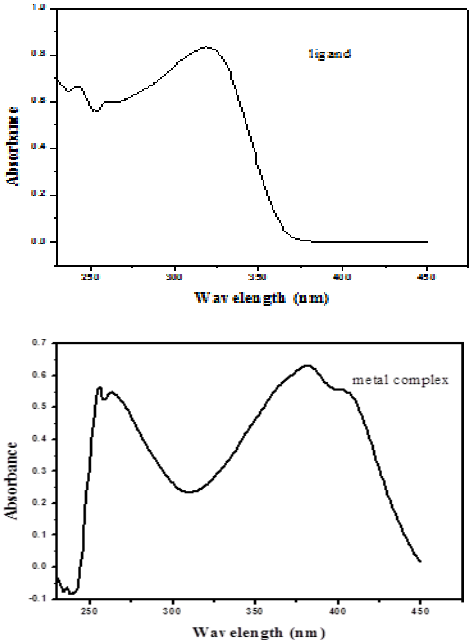


**Fig. 2. Electronic spectrum of ligand and complex**

*3.3. Proton NMR spectrum of the ligand*

$^1$H-NMR spectrum of the free hydrazone ligand recorded using $CDCl_3$ as solvent was assigned on the basis of observed chemical shift. The spectrum displayed a singlet due to an NH proton δ 9.0 ppm. The ligand showed a sharp singlet for azomethine (HC=N) at δ 8.87 ppm. Signals corresponding to the protons of benzene proton and thiophene proton of the ligand were observed as multiplets in the range δ 7.22-8.58 ppm. NMR spectrum of the ligand ascertained its formation as expected.

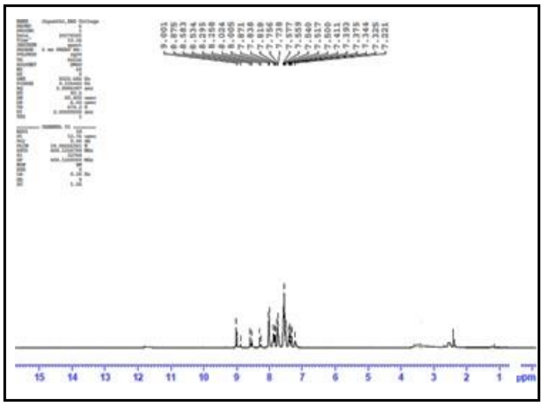


**Fig. 3. $^1$H NMR spectrum of the ligand**

*3.4. Thermal analysis of the complex*

Thermo-gravimetric analysis of the copper complex showed a single step decomposition pattern. It decomposed at 401°C with the formation of CuO. The percentage weight loss for the decomposition of the ligand was found to be 86.28 %. 13.72% that matches with the formation of CuO. The calculated value for the same is 13.99%. Thus it confirmed the compositon of the complex.

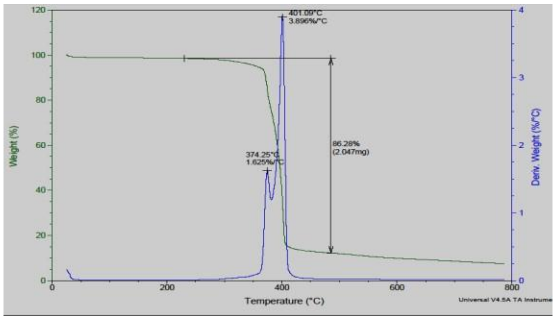


**Fig. 4.TG-DSC spectrum of complex**

Based on the above spectral and micro analysis data, a four coordinate square planar geometry has been proposed for the complex with 1:1 metal to ligand stoichiometry. The proposed structure of the complex is given below.

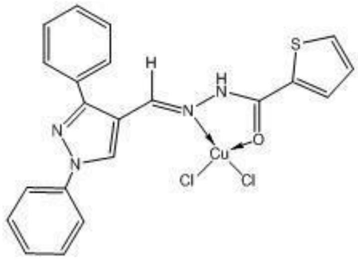


**Fig. 5. Proposed structure of copper complex**

### *3.5. DNA cleavage study*

Generally DNA damage is indicated by the conversion of supercoiled form of plasmid DNA to circular form. To check the role of synthesized complex on DNA breakage, plasmid DNA damage assay was performedand 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. 6). 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.

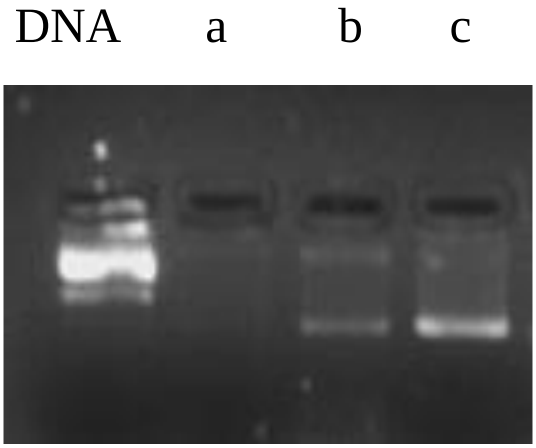


**Fig. 6. pBR322 plasmid DNA cleavage using different concentration of complex (a-10 μM, b-20 μM and c-30 μM)**

## 4. CONCLUSION

Interesting coordination modes of hydrazone and their biological perspective provoked us to synthesize new copper hydrazone complex by using the ligand prepared from 1,3 diphenyl pyrazole-1H 4 carbaldehyde andthiophene carboxylic hydrazide (L). The ligand was characterised by FT-IR, UV-visible and NMR spectral method. The elemental analysis data of the ligand and the complex are in good agreement with the proposed molecular formulae. The presence of NH stretching vibration and reduction in the C=N and C=O stretching frequencies suggest the neutral bidentate coordination of the ligand in copper complex. The DNA cleavage studies showed that the complex has the potential to cleave DNA. Thus cytotoxic potential of the complex and mechanism of inducing apoptosis by oxidative pathway can further be analysed.

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