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IN VITRO CYTOTOXICITY STUDIES OF A NOVEL SCHIFF BASE LIGAND DERIVED FROM 4-HYDROXY BENZOYL HYDRAZIDE AND 2-ACETYL BENZOFURAN AND ITS CU (II), NI (II) METAL COMPLEXES.

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Abstract: In vitro cytotoxicity studies of a novel Schiff base ligand derived from the condensation of 4-hydroxy benzoyl hydrazide and 2-acetyl benzofuran (HBZABF) and its Cu (II), Ni (II) complexes are reported. The IR and UV-visible spectral data suggest the formation of the Schiff base ligand and its metal complexes. The percentages of carbon, hydrogen and nitrogen present in the Schiff base ligand and complexes are confirmed by Elemental analysis. The analytical results suggest that the Schiff base behaves as tridentate ONO donor ligand and coordinates with Cu (II) and Ni (II) ions in octahedral geometry. The ligand and its complexes are screened for their cytotoxicity. Cytotoxicity studies reveal that the Schiff base complex of copper exhibits considerable activity than the free Schiff base ligand. Copper complex of HBZABF showed 100 % cytotoxicity at a concentration of 200 **µg/ml.** Copper complex exhibited high cytotoxicity even in lower concentrations and it can be used as a promising anticancer drug after further investigations.

***IndexTerms* - 4-Hydroxy benzoyl hydrazide, 2-Acetyl benzofuran, Schiff base, Cytotoxicity**

I. INTRODUCTION

Schiff bases are compounds formed through the condensation of primary amines with aldehydes or ketones and are characterized by the presence of an imine group (–C=N–). Schiff bases are represented by the general formula of RHC=N-R′ where R and R′ are alkyl, aryl, cycloalkyl or heterocyclic groups [7, 13]. Schiff bases were first synthesized in 1864 by Hugo Schiff. In his honor the compounds containing azomethine group are named as Schiff bases. Schiff bases act as potential ligands in coordination chemistry since they contain an active imine [-C=N-] bond having great affinity for metallic ions [16, 9]. The Schiff bases play a crucial role in various fields such as organic chemistry, coordination chemistry and biochemistry due to their diverse chemical reactivity and ability to form stable complexes with metal ions. Schiff base metal complexes act as homogeneous and heterogeneous catalysts in many reactions [10,4,5].The various applications of Schiff base metal complexes reported earlier include biological applications like anticancer[21], antibacterial[14], antifungal[8], antituberculosis[23], antiviral[15] , antioxidant[17] and anti-inflammatory [18], activities. Recent studies revealed that Schiff base metal complexes are used as photosensitizers in dye sensitized solar cells [11,19].

A particular example of Schiff bases are hydrazones derived from hydrazides which are monosubstituted hydrazine derivatives that contain their specific -NH-NH- nitrogen bridge and also a carbonyl or sulfonyl group linked directly to one of the nitrogen atoms (R-NH-NH2 where R is -C(=O)- or -S(=O)-). Hydrazones are characterized as having a basic structure $R_1R_2C=NNR_3R_4$ [25,20]

The Imine group present in Schiff base plays an important role for their biological activities [6, 12]. Recently the interaction between transition metal complexes and DNA has attracted much interest due to their importance in cancer therapy, design of new types of pharmaceutical molecules and molecular biology [3,24].The complexes of transition elements with Schiff bases have wide applications in food industry, dye industry, catalysis, fungicidal, agrochemical, anti-inflammable activity, antiradical activities and biological activities [1,2]. Heterocyclic Schiff base ligands and their complexes possess great importance due to their pharmacological properties [22].

II. RESEARCH METHODOLOGY

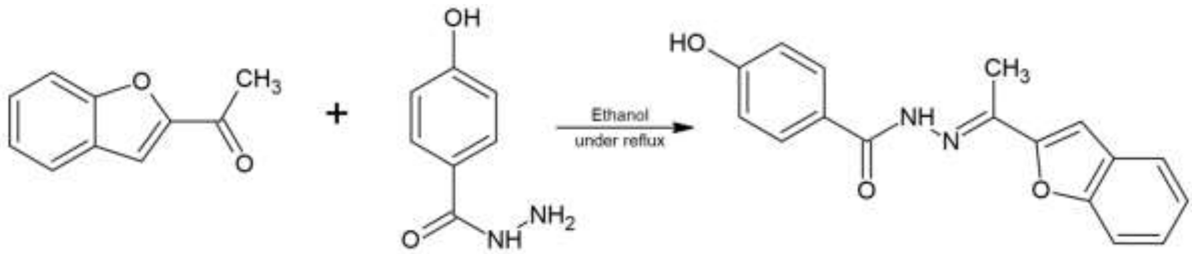
The analytical grade reagents and chemicals including 4-hydroxy benzoyl hydrazide, 2-acetyl benzofuran, ethanol, metal acetates (Cu, Ni) were purchased from Sigma Aldrich. All chemicals were used without further purification.

2.1 Synthesis of the Schiff base ligand HBZABF

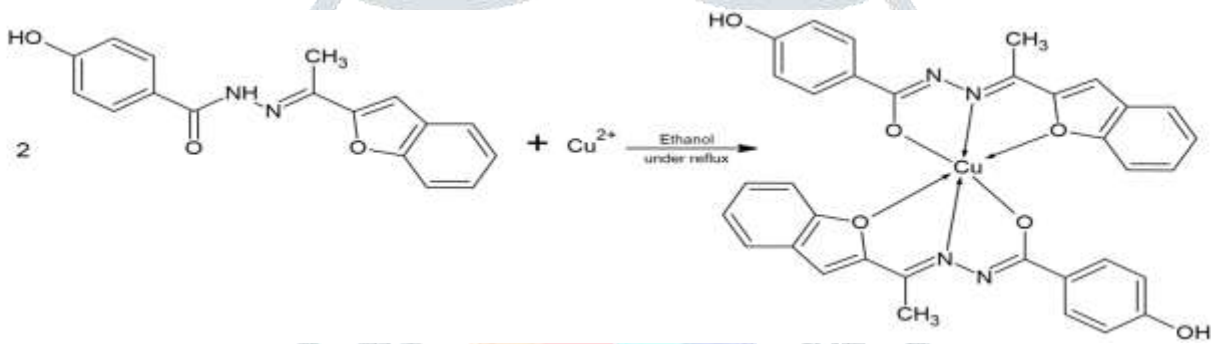
4-hydroxy benzoyl hydrazide (1mmol) was dissolved in 20 ml methanol .To this solution2- acetyl benzofuran (1 mmol) in 10 ml methanol was added drop wise with stirring. The resulting mixture was refluxed for 3 hours. The colorless solution was cooled overnight, filtered and dried.

2.2 Synthesis of the metal complexes

The Schiff base ligand (1mmol) was dissolved in methanol. To the refluxing solution of the Schiff base ligand metal acetate (Cu (II), Ni (II)) (0.5mmol) was added. The resulting solution was refluxed for 5 hours. The mixture was cooled overnight. The separated solid was filtered, washed and dried.



Scheme 1: Synthesis of HBZABF



Scheme 2: Synthesis of HBZABF Copper complex

III. In Vitro Cytotoxicity Studies

The test compound was studied for short term in vitro cytotoxicity using Dalton's Lymphoma Ascites cells (DLA). The test compounds HBZABF, $(HBZABF)_2Cu$ & $(HBZABF)_2Ni$ were dissolved in DMSO and concentration range between 200 **μ**g/ml to 5**μ**g/ml was used for the study. The tumor ce1ls aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal cell line. Cell viability was determined by trypan blue exclusion method. A viable cell suspension ($1x10^6$ cells in 0.lml) was added to tubes containing various concentrations of the test compounds and the volume was made up to l ml using phosphate buffered cell line (PBS). The control tube contained only cell suspension. These assay mixtures were incubated for 3 hours at 37^0C. Further cell suspension was mixed with 0.lml of l% trypan blue and kept for 2-3 minutes and loaded on a haemocytometer. Dead cells take up the blue colour of trypan blue while live cells do not take up the dye. The numbers of stained and unstained cells were counted separately.

$$\%\ \text{cytotoxicity} = \frac{No.of\ live\ cells}{No.of\ live\ cells + No.of\ dead\ cells} \times 100$$

IV. RESULTS AND DISCUSSION

3.1 Physicochemical Analysis

Elemental analysis was performed for the Schiff base ligands and the metal complexes. The experimental data was in close agreement with the theoretical values. This suggests complex formation.

Sl. No	Compound	Molecular Formula	Colour	Melting point	% of C Found (Calc.)	% of H Found (Calc.)	% of N Found (Calc.)
4	HBZABF	$C_{17}H_{14}N_2O_3$	White	254	69.19 (69.38)	4.66 (4.79)	9.42 (9.52)
5	$(HBZABF)_2Cu$	$C_{34}H_{26}N_4O_6Cu$	Black	>275	62.72 (62.81)	4.00 (4.03)	8.52 (8.62)
6	$(HBZABF)_2Ni$	$C_{34}H_{26}N_4O_6Ni$	Black	>275	63.15 (63.29)	4.03 (4.06)	8.69 (8.68)

Table 1: Physicochemical data of the ligands and metal complexes.

3.2 FTIR Spectral analysis

In the FTIR spectra of the Schiff base ligand HBZABF the bands due to -OH and -NH were observed at 3394 cm^{-1} and 3265 cm^{-1} respectively. The azomethine band was observed at 1612 cm^{-1}. The band due to –C=O group appeared at 1784 cm^{-1}.
In metal complexes the band due to azomethine group was shifted to lower wavenumber (20-25 cm^{-1}) suggesting the coordination of azomethine nitrogen atom to the metal. The strong vibrational band of carbonyl group of the ligand showed a shift from 1784 cm^{-1} to lower frequency again supported the coordination of the lone pair of electrons of oxygen atom of C=O group. Bands due to M-N bonds were observed 558-586 cm^{-1} in complexes confirming complex formation as these bands were absent in Schiff base ligands. Thus the IR data strongly suggest the monobasic tridentate ONO donor behaviour of each Schiff base unit.

Compound	**ν(C=O)**	**ν(C=N)**	**ν(O–H)**	**ν(N–H)**	**ν(M–N)**
HBZABF	1784	1612	3394	3265	--
$(ABZHBF)_2Cu$	1767	1592	3275	-	586
$(ABZHBF)_2Ni$	1764	1587	3271	-	574

Table 2: FTIR Spectral data of the ligand and metal complexes.

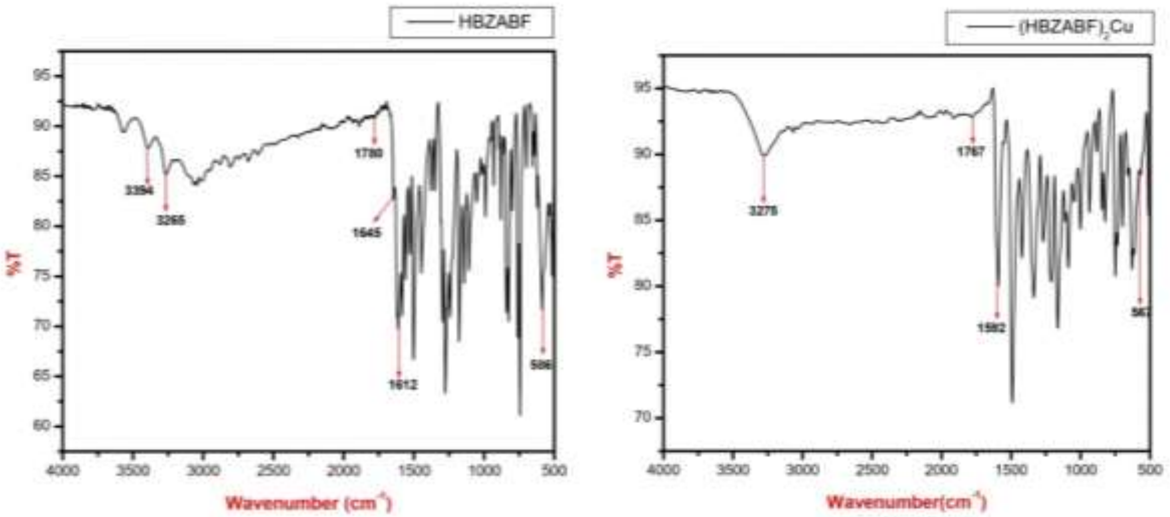


Fig.1: IR Spectra of HBZABF and $(HBZABF)_2Cu$ Complex

3.3 UV-Visible Spectral Analysis

The UV visible spectral analysis of the ligand and the complexes were carried out in DMSO solvent. In HBZABF maximum absorbance was observed at 402nm and 505 nm due to intra ligand pi to pi* and n to pi* transitions. In metal complexes maximum absorbance band was shifted to higher wavelength region supporting complex formation. A broad absorption band at 529nm range was detected in metal complexes which may be due ligand to metal charge transfer LMCT transition.[28-29]

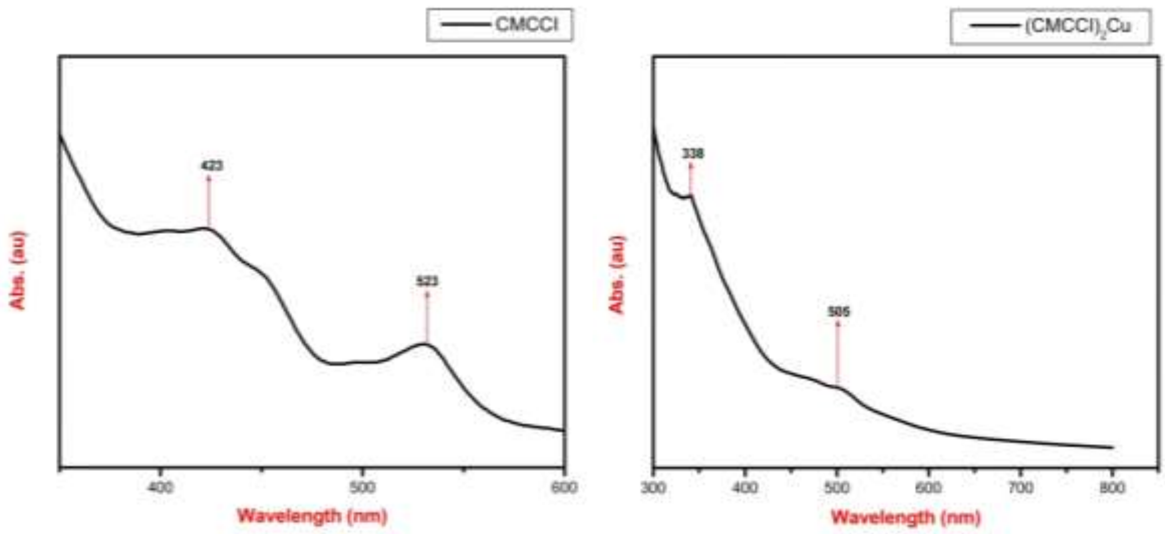


Fig.2: UV-visible Spectra of HBZABF and $(HBZABF)_2Cu$ Complex

3.6 In vitro cytotoxicity Studies

In vitro cytotoxicity studies using Dalton's Lymphoma Ascites cells (DLA) shows that percentage cell death is maximum in the case of Copper complex. Furthermore, cytotoxicity effect of all the compounds increases with increase in concentration. Cobalt and nickel complexes exhibit lower cytotoxicity compared to the Schiff base ligand. From the data it is evident that the copper complex show remarkable cytotoxicity even at lower concentrations and can be used as a potent anticancer drug after further investigations.

Drug concentration (µg/ml)	**HBZABF**	**$(HBZABF)_2Cu$**	**$(HBZABF)_2Ni$**
5	**-**	**5.26±0.8**	**-**
7.5	**-**	**15.1±1.7**	**-**
12.5	**4.10±0.4**	**40.3±2.2**	**3.51±0**
25	**5.68±0.5**	**47.6±1.3**	**4.46±0**
50	**8.97±0.8**	**61.5±2.6**	**4.68±0.4**
100	**13.0±2.0**	**79.8±1.8**	**6.68±0.9**
150	**20.5±2.1**	**92± 5.5**	**7.5±1.8**
200	**25.7±1.8**	**100±0**	**12.5±1.5**

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