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Synthesis, Characterization, Cytotoxic, Anticancer and Antimicrobial Studies of Novel Schiff Base Ligand Derived From Vanillin and Its Transition Metal Complexes

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Abstract

A new series of Co(II), Ni(II), Cu(II) and Zn(II) complexes of the Schiff base derived from Vanillin and acetoacetanilide with ethylenediamine has been synthesized. These compounds have been characterized based on their elemental analyses, conductivity measurements, infrared and electronic spectra. The Schiff base ligand has been further identified by ^{1}H NMR. The ligand and its metal complexes were tested its cytotoxic activities and found that the copper complex to be higher Inhibitory Concentration (IC_{50}) value around 49 µ/ml. DLA cell induced solid tumour model and EAC cell induced ascites tumour model were mainly used for antitumor studies. Copper complex administrated at different concentrations in Swiss albino mice to access increases in the survival rate and the life span of Ascites tumour enduring mice in a concentration dependent manner. Antimicrobial activities of the Schiff base ligand and their metal complexes reveals that the Schiff base transition metal complexes show significant activity against some fungi and bacteria.

Keywords: Schiff base ligand, cytotoxic activities, Daltons Lymphoma Ascites cell, Ehrliche`s Ascites Carcinoma cell, antitumor study

INTRODUCTION

Schiff bases have been known since 1864 when the famous scientist, Hugo Schiff reported the condensation product of primary amines with carbonyl compounds [1]. Compounds containing an azomethine group (-CH=N-), known as Schiff bases are easily formed by the condensation reaction of a primary amine with a carbonyl compound (aldehyde or ketone compounds)[2,3]. Schiff bases of aliphatic aldehydes are relatively unstable and are readily polymerized while those of aromatic aldehydes, having an effective conjugation system, are more stable[4]. Schiff base could be applied in different areas such as separation processes, metallic deactivation, bioinorganic chemistry, catalysis, electro chemistry and environmental chemistry[5,6]. Microwave assisted preparation of a series of Schiff bases via efficient condensation of salicylaldehyde and aryl amines without solvent is described as a high yield and an eco-friendly method in organic synthesis[7]. Schiff bases find application in the field of agriculture (as pesticide) and in medicine with their highly effective antibacterial and anticoagulant activities [8]. Its metal complexes continue to attract many researchers because of their wide applications in various industrial, analytical and pharmaceutical fields [9,10]. e.g. in the treatment of cancer [11], as bactericides [12], antiviral agents [13], fungicides [14], and for other biological properties[15].

Literature review made known that the transition metal complexes of Schiff base show antitumour property[16]. Schiff base complexes exercise their biological activity in mamalian cells by inhibiting ribonucleotide reductase, a essential enzyme in the synthesis of DNA precursors[17]. Iron and copper complexes have shown that they can be more active in the inhibition of DNA synthesis than the free ligand itself [18]. Earlier studies showed that the copper(II) complex of 2-formylpyridine thiosemicarbazone is very powerful antitumour agent than the free ligand[19]. Petering[20] reported that the Copper(II) ion itself have any antitumour activity, but will act as an inhibitor of tumour growth in the chelated or complexed form. Even though, the definite pathway by which the copper chelate reduce the cancer growth is not known, it is supposed that this will be on the basis of structure activity relationship as in the case of cisplatin[21] (i.e. DNA intercalation).

The review also exposed that Schiff base of diamine were extensively studied, but not many, which were studied, show considerable biological activity. Furthermore, complexes of Schiff bases ligand derived from vanillin and acetoacetanilide with ethylenediamine and their transition metal complexes are take up here. This compound can be expected to act as excellent chelating agents because of the presence of two azomethine groups. Metal ion of the first transition series can most likely coordinated with this dianionic tetradentate ligand. Metal ion and the ligand react in 1:1 molar ratio; there is a chance of anion coordination as well. Tetradentate (N_2O_2) Schiff base ligand Vanillin-(1,2-ethylenediimin)ethylacetoacetanilide and its Co(II), Ni(II), Cu(II) and Zn(II) metal complexes were synthesized and determined *in vitro* cytotoxicity. The antitumour studies were carried out using the ligand Vanillin-(1,2-ethylenediimin)ethylacetoacetanilide and its Cu(II) complex. The ligand and their metal complexes were screened *in vitro* for their antifungal activity against three pathogenic fungi, *Fusarium semitectum, Aspergillus niger* and *Aspergilus flavus*, by the agar plate method. We also studied the capability of these compounds for *in vitro* antibacterial activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis*.

EXPERIMENTAL SECTION

Materials and methods

The metal salts used in this study were BDH AnalaR quality and purchased from E. Merck. For the preparation

of ligands, Vanillin and acetoacetanilide with Ethylenedaimene were used. Mainly chlorides and acetate of Co(II), Ni(II), Cu(II) and Zn(II) salts were used for the synthesis of Schiff base metal complexes. The solvents used for the synthesis, extraction and recrystalization of the ligands and the complexes were ethanol, methanol, chloroform, DMF, DMSO, petroleum ether, diethyl ether, etc. Commercially available solvents, like ethanol and methanol were purified by standard methods[22,23,24]. Others were E. Merck reagent grade and were used as such. The solvents, such as methanol and dimethylformamide used for spectral and conductivity measurements were of spectroscopic grade.

Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel F_{254} plates from Merck and the compounds were visualized by exposure to UV light and kept in iodine chamber. Chromatographic columns of 70-230 mesh silica gels were used for separation. Melting point was determined using a Fisher-Johns apparatus. Elemental analysis for C, H, N and S were done by making use of VarioEL-III CHNS analyzer. The molar conductance of the complexes in DMF was measured using a Sybron-Barnstead conductometer (Meter-PM.6, E = 3,406). The IR spectrum was recorded on a Jasco FTIR-4100 Spectrophotometer by using KBr pellet. The electronic spectrum in the range of 200-900 nm was recorded using Jasco UV-Vis spectrophotometer. ^{1}H NMR spectrum in dimethylsulphoxide (DMSO-d_6) was recorded on a 500 MHz Brucker AV 500 FT NMR spectrometer.

Synthesis of Vanillin-(1,2-ethylenediimine)acetoacetanilide (VEAc)

Vanillin (0.025 mol) and acetoacetanilide (0.025 mol) in minimum amount of methanol was added to ethylenediamine (0.025 mol) in methanol in 250 ml round bottom flask. Added a few drops of mineral acid as dehydrating agent. The mixture was allowed to reflux for 3 hour. After the reaction completed, the mixture was cooled in ice bath and the yellow product, which was first formed, was filtered and washed with ethanol and dried over anhydrous calcium chloride (Fig. 1). Yellowish crystalline solid; yield 80 %; m. p: 180^{0}C; Solubility: DMSO, DMF; UV-Vis λ_{max}: 286 nm, 436 nm; IR: ν = 1610 cm^{-1} (C=O), ν = 1589 cm^{-1} $(C=N)_{azomethine}$, ν = 1517 cm^{-1} $(C=N^I)_{azomethine}$, ν = 3317 cm^{-1} (-OH), ν = 2049 cm^{-1} (-NH), ν = 3110 cm^{-1} (Ar^I), and ν = 3094 cm^{-1} (Ar^{II}); ^{1}H NMR (DMSO-d_6): δ = 7.61-7.19 (m, 4H, Ar^I), δ = 7.22-7.04 (m, 3H, Ar^{II}), δ = 5.35 (s, 1H, -OH), δ = 7.23 (s, 1H, -NH), δ = 3.24 (s, 1H, -CH_2) and δ = 8.54 (s, 1H, -CH=N). (**Fig. 1**)

Synthesis of metal complexes

A methanolic solution of the metal salt (0.005 mol in 20 ml) was added to a solution of the ligand (0.005 mol in 20 ml) taken in dimethyl sulphoxide (DMSO) and the mixture were refluxed for 4 h. It was then cooled and kept allowed to evaporate. The solid complexes formed was filtered off, washed several times with petroleum benzene and finally with methanol. It was then dried over anhydrous calcium chloride (**Fig. 2**).

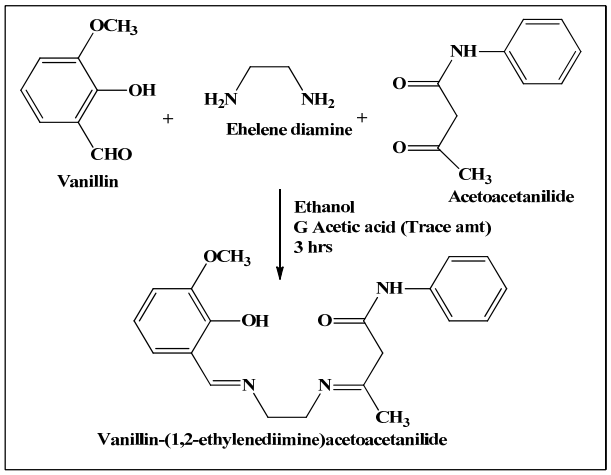

Fig 1: Preparation of Schiff base ligand, Vanillin-(1,2-ethylenediimine)acetoacetanilide (VEAc)

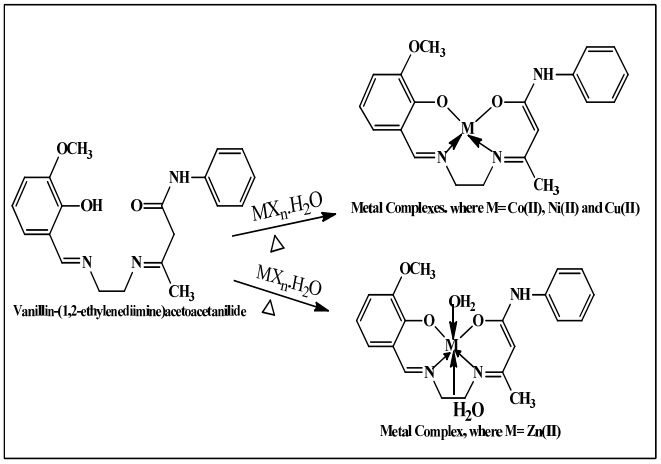

Fig 2: Scheme of the synthesis of VEAc Schiff base complexes of Co(II), Ni(II), Cu(II) and Zn(II)

[Vanilline-(1,2-ethylenediamine)acetoacetanilide]Co(II) complex

Dark green solid; yield 51 %; m.p: 300^{0}C; μ_{eff} = 4.9 MB; Λ_m = 45.27 Ω^{-1} mol^{-1} cm^{-2}; IR: ν = 1590 cm^{-1} (C=O), ν = 1580 cm^{-1} $(C=N)_{azomethine}$, ν = 1510 cm^{-1} $(C=N^I)_{azomethine}$, (-NH), ν =1124 (CH=C-OH), ν = 3110 cm^{-1} (Ar^I), and ν = 3094 cm^{-1} (Ar^{II}), ν =410cm^{-1} (-O-M), ν = 512 cm^{-1} (-N-M) ;

[Vanilline-(1,2-ethylenediamine)acetoacetanilide]Ni complex

Dark green solid; yield 55 %; m.p: 298^{0}C; μ_{eff} = 3.26 MB; Λ_m = 56.27 Ω^{-1} mol^{-1} cm^{-2}; IR: ν = 1594 cm^{-1} (C=O), ν = 1580 cm^{-1} $(C=N)_{azomethine}$, ν = 1500 cm^{-1} $(C=N^I)_{azomethine}$, ν = 1121 (CH=C-OH), ν = 2049 cm^{-1} (-NH), ν = 3110 cm^{-1} (Ar^I), and ν = 3094 cm^{-1} (Ar^{II}), ν = 405cm^{-1} (-O-M), ν = 503 cm^{-1} (-N-M) ;

[Vanilline-(1,2-ethylenediamine)acetoacetanilide]Cu(II) complex

Dark green solid; yield 48 %; m.p: 275^{0}C; μ_{eff} = 2.1 MB; Λ_m = 39.27 Ω^{-1} mol^{-1} cm^{-2}; IR: ν = 1590 cm^{-1} (C=O), ν = 1574 cm^{-1} $(C=N)_{azomethine}$, ν = 1503 cm^{-1} $(C=N^I)_{azomethine}$, ν = 2049 cm^{-1} (-NH), ν = 1120 (CH=C-OH), ν = 3110 cm^{-1} (Ar^I), and ν = 3094 cm^{-1} (Ar^{II}), ν = 418cm^{-1} (-O-M), ν = 520 cm^{-1} (-N-M) ;

Table 1: Physical properties of ligand (VEAc) and metal complexes

Compounds	Melting point (^{0}C)	CHN Analysis found (Calculated) %				Molecular Structure	Molar Conductance Ω^{-1} mol^{-1} cm^{-2}
		%C	%H	%N	% metal		
LH_2 (VEAc)	180	68.9 (67.9)	6.0 (6.5)	12.0 (11.8)	--	$C_{20}H_{23}N_3O_3$	---
CoL	<300	59.0 (58.5)	5.0 (5.1)	9.9 (10.2)	15.0 (14.4)	$CoC_{20}H_{21}N_3O_5$	45.27
NiL	298	57.1 (58.5)	5.8 (5.1)	11 (10.2)	14.0 (14.3)	$NiC_{20}H_{21}N_3O_3$	56.27
CuL	275	56.0 (57.8)	5.9 (5.1)	11 (10.1)	15.9 (15.1)	$CuC_{20}H_{21}N_3O_3$	39.00
$ZnL(H_2O)_2$	265	54.0 (53.0)	6.0 (5.5)	10 (9.2)	15.0 (14.2)	$ZnC_{20}H_{25}N_3O_5$	50.01

Table 2: Observed electronic transitions and magnetic moment data of metal complexes

Complex	Eectronic spectral data		Magnetic moment (B.M)	Geometrical structure
	Transition	Assignment (cm^{-1})		
CoL	$^4A_2 \rightarrow {}^4T_2$ $^4A_2 \rightarrow {}^4T_1$ $^4A_1 \rightarrow {}^4T_1$(P)	8110 7792 16255	4.9	**Tetrahedron**
NiL	$^3T_1 \rightarrow {}^3T_2$ $^3T_1 \rightarrow {}^3A_2$ $^3T_1 \rightarrow {}^3T_1$(P)	8500 7549 14250	3.26	**Tetrahedron**
CuL	$^2T_2 \rightarrow {}^2E$	23800	2.1	**Distorted Tetrahedron**
$ZnL(H_2O)_2$	------	------	-----	**Octahedron**

[Vanilline-(1,2-ethylenediamine)acetoacetanilide]Zn(II) complex

Dark green solid; yield 57 %; m.p: 265^{0}C; Λ_m = 50.27 Ω^{-1} mol^{-1} cm^{-2}; IR: ν = 1589 cm^{-1} (C=O), ν = 1560 cm^{-1} $(C=N)_{azomethine}$, ν = 1503 cm^{-1} $(C=N^I)_{azomethine}$, ν = 2049 cm^{-1} (-NH), ν = 1129 (CH=C-OH), ν = 3110 cm^{-1} (Ar^I), and ν = 3094 cm^{-1} (Ar^{II}), ν = 401cm^{-1} (-O-M), ν = 492 cm^{-1} (-N-M); (**Table. 1 &2**)

Anticancer Studies

Synthesis and evaluation of anticancer potential of drug

50 mg of the compound was dissolved in 1 ml of dimethyl sulphoxide (DMSO), for *in vitro* studies. *An in vivo study* 50 mg of drug was first dissolved in 1 ml DMSO and further it was diluted using distilled water to desired concentration.

Adayar Cancer Institute, Chennai provided essential Ehrlich Ascites Carcinoma (EAC) cell lines and Dalton's Lymphoma Ascites (DLA) cell and propagated as transplantable tumours in the peritoneal cavity of BALB/C mice. L929 (mouse lung fibro blast) cell line was obtained from National Centre for Cell Sciences, Pune.

The experiment were carried out the laboratory mice Swiss albino female (20-25 g) and were obtained from the Small Animal Breeding Station, Mannuthy, Thrissur, Kerala and they were kept under standard conditions of temperature and humidity in animal house of Amala Cancer Research Centre, Trissur Kerala. The animals were provided with standard mouse chow (Sai Durga Feeds and Foods, Bangalore, India) and water *ad libitum*. All the animal experiments in this study were carried out with the prior approval of the Institutional Animal Ethics Committee (IAEC) and were conducted strictly according to the guidelines of CPCSEA constituted by the Animal Welfare Division, Government of India.

Mouse lung fibroblast (L929 cells) were cultured in DMEM medium supplemented with FBS (10% v/v), streptomycin (100 µg/ml) and penicillin (100 U/ml) and kept at 37^{0}C in an incubator with 5% CO_2. Dalton's Lymphona Ascites (DLA) and Ehrlich's Ascites Carcinoma (EAC) cells maintained in the intraperitoneal cavity of mouse were used for the study.

Trypan blue exclusion method:

The test compounds were studied for short-term *in vitro* cytotoxicity using Dalton's lymphoma ascites cells (DLA). The tumour cells aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal saline. Cell viability was determined by Trypan blue exclusion method [25].

Metal complexes toxicity:

24 Swiss albino mice were divided into 4 groups (6 animals/group). 5 mg/kg, 10 mg/kg, and 15 mg/kg, 20 mg/kg, treated group 1, group 2 group 3 and group 4 respectively. The drug was administrated once daily (i.p.) and continued for 5-6 weeks to determine their mortality rate.

Treatment of Vanillin-(1,2-ethylenediimine)acetoacetanilide copper complex

On the survival rate of ascites tumour bearing mice:

Mice (female, 6–8 weeks old) weighing 26–30 g were divided into 4 groups of 6 animals each. Viable EAC cells 106 in 0.1 ml of phosphate buffered saline (PBS) were injected in to the peritoneal cavity. Group 1: control, group 2: 5 mg/kg, treated, group 3: 10 mg/kg, treated, group 4: 15 mg/kg, treated, and group 5: standard drug (cyclophosphamide), treated.

Drug and cyclophosphamide were given by intraperitoneal injection from the first day of tumour induction. The death pattern of animals due to tumour burden was noted and the percentage of increase in life span was calculated as, % ILS= (T-C/C) ×100, where T and C are mean survival of treated and control mice, respectively.

Antifungal activity

The isolation of fungi was done by dilution plate method [26]. Selected and isolated fungi were maintained on potato dextrose agar plates at 4°C for further study. The antifungal actions of the ligands, Schiff base metal complexes, fungicides (bavistin and emcarb), and the control dimethyl sulfoxide (DMSO) were screened using the plate poison technique [27]. Seven day-old cultures of *Fusarium semitectum*, *Aspergillus niger* and *Aspergillus flavus* were used as test organisms. A stock solution of 0.5 mg/ml was made by dissolving 50 mg of each compound in DMSO (100 ml). The sterilized medium with the added stock solution was poured into 90 mm sterile petri-plates and allowed to solidify. They were inoculated with a 5-mm actively growing mycelia disc and incubated at 27°C for 72 h. After 72 h of inoculation, the percent reduction in the radial growth diameter over the control was calculated. The growth was compared with dimethylsulfoxide as the control.

Antibacterial study

The in vitro antibacterial activity of the ligand and its corresponding metal complexes was investigated against the standard strains of two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. In order to compare the results, Vancomycin (30 mg/disc), Colistin (2 mg/disc) and Nalidixic acid (30 mg/disc) were used as standard antibacterial drugs. Determination of the antibacterial activity was carried out by the paper-disc diffusion method. The compounds were dissolved in DMSO at 40 mg/ml concentration. Muller Hinton broth was used for preparing the basal media for the bioassay of the organisms. A lawn culture from a 0.5 MacFarland suspension of each strain was prepared on Muller Hinton agar. Blank paper discs (6.4 mm diameter) were saturated with a solution of the test compounds and placed on the surface of the agar plates. On one paper disc only, DMSO was poured as a control. The plates were incubated at 37^0C for 24 h. The inhibition zone diameters around each disc were measured in mm.

RESULTS AND DISCUSSION

Characterization of the Schiff base ligand and its metal complexes

The ligand and complexes were characterized based on their elemental analysis, magnetic susceptibility measurements, IR, UV-Vis and ^{1}H NMR spectral studies.

Analytical data are given in the Table. 1, which, confirmed the exact composition of the ligand and complexes. The electronic absorption spectrum of the ligand showed two bands at 286 and 436 nm. The first one may be assigned to intra-ligand π–π* transition, which is nearly unchanged on complexation, whereas the second band may be assigned to the n–π*.

The molar conductivities of 10^{-3} M of the complexes (dissolved in DMF) at room temperature were measured (Table 1). The results were in the range 40-50 Ω^{-1} mol^{-1} cm^2 for Co(II), Ni(II), Cu(II) and Zn(II) complexes. Conductivity measurements reveal that all the metal complexes have conductivity values in the range characteristic for non-electrolytic nature.

In the IR spectra of the complexes a new bands are observed around 1124 cm^{-1}, indicating the enolization of –CH_2-C=O to –CH=C-OH and subsequent coordination through the deprotonated oxygen. Two bands of medium intensity at around 1580 and 1511 cm^{-1} in the spectrum of ligand may be assigned to (-C=N) and (-C=N^I)[28,29,30]. However, the spectra of all the complexes, this band is found to be shifted a lower frequency region by a few cm^{-1}, indicating the participation of azomethine nitrogen in coordination. A broad band at ~ 3317 cm^{-1} in the spectrum of the ligand is attributed to the hydroxyl stretching modes. In addition, medium bands around 410 and 512 cm^{-1} in the spectra all the complexes may be assigned to $\nu_{(M-N)}$ [28,31,32,33] and $\nu_{(M-O)}$, respectively.

The electronic spectra of the Co(II) complex showed three bands at 8110, 7792, 16255 cm^{-1}. These bands are assigned to the transitions $^4A_2 \rightarrow {}^4T_2$, $^4A_2 \rightarrow {}^4T_1$ and $^4A_1 \rightarrow {}^4T_1$(P) respectively; the CoII complex also showed magnetic moment at 4.9 B.M., which indicates the presence of Co(II) complex in tetrahedral geometry. The electronic absorption of Ni(II) complex showed three bands at 8500, 7549 and 14250 cm^{-1} (Table 2). These bands are assigned to the transitions $^3T_1 \rightarrow {}^3T_2$, $^3T_1 \rightarrow {}^3A_2$ and $^3T_1 \rightarrow {}^3T_1$(P) respectively[34,35,36], the magnetic moment of Ni(II) complex was 3.2 B.M. These results suggested the presence of tetrahedral geometry for Ni(II) complex. The electronic spectra of the Cu(II) complex showed one band at 23800 cm^{-1}, which are assigned to $^2T_2 \rightarrow {}^2E$ transition, and the magnetic moment of Cu(II) complex was 2.1 B.M. Both the electronic spectra and the magnetic values proposed the presence of the distorted tetrahedral geometry for Cu(II) complex. On the basis of the above observation and spectral data, it is suggested that the Co(II), Ni(II) and Cu(II) complexes show tetrahedral geometry structures; however, the Zn(II) complex shows octahedral geometry structure.

Based on the above data the probable structures of the ligand and its metal complexes are given below (**Fig. 3**)

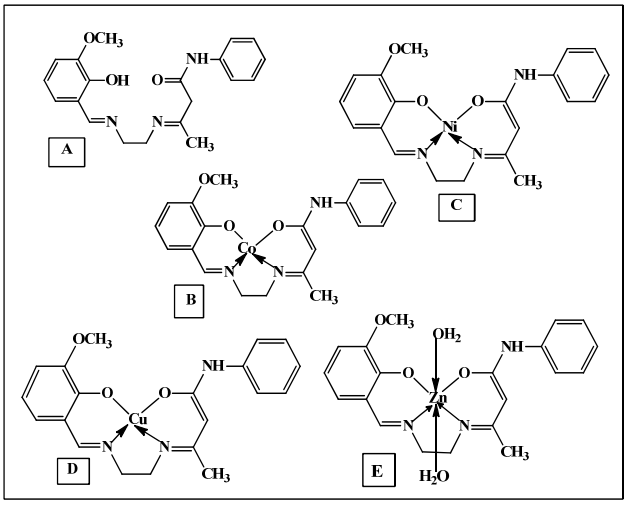

Fig 3: Structures of Schiff base ligand (VEAc) and its complexes. A: Schiff base ligand; B: Co complex; C: Ni complex; D: Cu complex; E: Zn complex

Short-term *in vitro* cytotoxic analysis:

The Schiff base ligand, VEAc and its Co(II), Ni(II), Cu(II) and Zn(II) complexes exhibit striking cytotoxic activity for DLA cell line (Table 1). The copper complex showed highest activity and the concentration required for 50% death (IC_{50}) was found to be 49 µg/ml. (**Table. 3**) (**Fig. 4**)

Toxicity studies of the drug:

The results of toxicity studies of copper complex on 24 Swiss albino mice, 4 groups, at four concentrations (20, 15, 10 and 5 mg/kg) showed that 20 mg/kg was slightly toxic to the animals. Therefore, this concentration was avoided and 15, 10 and 5 mg/kg were only selected for *in vivo* studies, as they were nontoxic to the animals. (**Table. 4**)

Table 3: Percentage of cytotoxicity of VEAc and complexes

Concentration (µg/ml)	Percentage of Cytotoxicity				
	Complexes				Ligand (VEAc)
	Co	Ni	Cu	Zn	
200	70	56	83	76	35
100	51	45	71	59	26
50	43	31	55	40	15
20	31	23	29	34	11
10	25	12	23	23	6

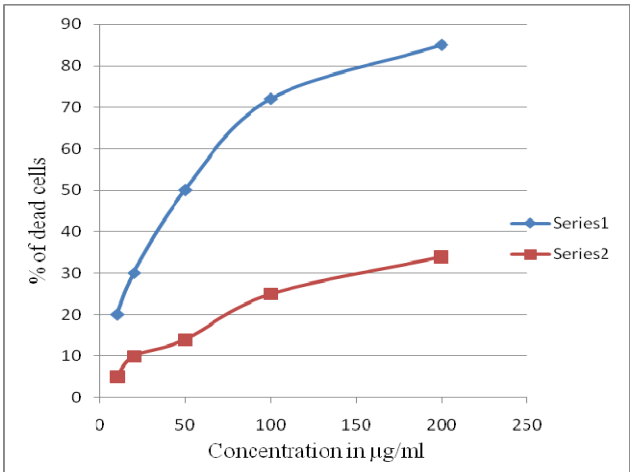

Fig 4: Cytotoxic action of VEAc ligand and its Copper complex

Table 4: Effect of copper complex of VEAc of survival rate of ascitis tumour enduring mice

Treatment (mg/kg)	Survival rate (Days)
Control	15.2
15	18.5
10	19.6
05	21
Standard*	25.8

*cyclophosphamide (10)

Treatment of copper complex on ascites tumour development:

The animals of the tumour control group survived for a period of 15.4 d. Those treated with cyclophosphamide survived for 25.8 d. The copper complex at 15, 10 and 5 mg/kg increased the survival rate of animals by 18.5, 19.6 and 21 d respectively (Table. 4). Thus the copper complex was found to be effective in increasing the average life span of the animals by 38.7, 28.9 and 21.7 %, respectively, at 5, 10 and 15 mg/kg doses (**Table. 5**)

Table 5: Effect of copper complex of VEAc on the life span rate of ascitis tumour enduring mice

Treatment (mg/kg)	Increase in Life span (%)
Control	--
15	21.7
10	28.9
05	38.7
Standard*	69.7

* cyclophosphamide (10)

The antitumour studies of Vanillin-(1,2-ethylenediimine)acetoacetanilide and its Co(II), Ni(II), Cu(II) and Zn(II) metal complexes and we got interesting and promising results due to the N-N-S and O-N-O donor system is a common feature for all compounds including ligand and metal complexes with carcinostatic potency. *In vitro* cytotoxicity studies on VEAc and its different transition metal complexes showed cytotoxicity against DLA cell lines. The copper complex showed highest cytotoxicity with an IC_{50} value of 49 µg/ml.

The present study we concluded that the copper complex of VEAc was found to be efficient against EAC-induced ascites tumour. The 5 mg/kg body weight was more effective than the other two concentrations (15 and 10 mg/kg b.wt) in both the cases and *in vitro* cytotoxic properties of the copper complex of VEAc suggest its potential use as an anticancer agent.

Antimicrobial activity:

The free Schiff base ligand and its respective metal chelates were screened against *F. semitectum A.niger* and *A. Flavus* fungi and *S aureus B. Cereus, P Aeruginosa* and *E. coli* bacteria to assess their potential antimicrobial action. It is clear from the antifungal screening data (Table. 6) that the Schiff base ligand itself was less reactive fungitoxic than chelated metal complexes. The bacterial screening results (Table.7) reveal that the free Schiff base ligand has more sensitivity for gram-positive than gram-negative bacteria. The biological activity of the complexes follows the order Cu(II) > Zn > Co(II) > and Ni(II). Metal complex was found to have higher biological activity than the parent Schiff base (Table. 6 and 7) towards both gram-positive and gram-negative bacteria. This indicates that the incorporation of metal ions in chelation can improve the biological activity of the parent organic compounds. (**Table. 6 & 7**)

The Schiff base ligand and their metal complexes were tested for their in vitro antimicrobial activity against two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. The inhibition zone diameter (mm/mg sample) values of the investigated compounds are summarized in Table. 7. A comparative study of inhibition zone diameter (mm/mg sample) values of the bis-Schiff base ligand and their metal complexes indicates that the metal complexes exhibit higher antimicrobial activity than the free Schiff base ligand (Table. 7).

Table 6: antifungal studues of VEAc and its metal complexes

Compounds	Fungi , % Inhibition (growth diameter in mm)		
	F. semitectum	***A.niger***	***A. Flavus***
Emcarb*	(00)100	(00)100	
Bavistin*	(00)100	(00)100	(00)100
DMSO (control)	(21)28	(28)30	(32)42
Ligand	(12)16	(12)13	(15)18
CoL	(23)25	(18)27	(15)24
NiL	(14)24	(23)26	(17)21
CuL	(19)33	(26)29	(24)39
$ZnL(H_2O)_2$	(20)29	(21)26	(18)26

* Conventional fungicides.

Table 7: Inhibition zones (mm) of complexes and ligand against bacterial strains

Compounds	Bacteria (D* mm)			
	Staphylococcus aureus	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas aeruginosa***
LH_2	8	7	6	6
CoL	12	13	11	10
NiL	8	13	11	8
CuL	15	16	14	12
$ZnL(H_2O)_2$	--	9	8	11
Vancomycin	18	--	17	--
Colistin	17	--	14	16
Nalidixic acid	20	21	24	--
DMSO	--	--	--	--

*(*D*) Diameter inhibition zone (in mm)

Such increased activity of the complexes can be explained on the basis of Tweedy's chelation theory [27,37,38]. Chelation reduces the polarity of the metal ion significantly because of the partial sharing of its positive charge with the donor group and also due to pi-electron delocalization on the whole chelate ring. The lipids and polysaccharides are some important constituents of the cell wall and membranes which are preferred for metal ion interaction. Apart from this, the cell walls also contain many phosphates, carbonyl, and cysteinyl ligands which maintain the integrity of the membrane by acting as a diffusion barrier and also provide suitable sites for binding. Furthermore, the decline in polarity increases the lipophilic nature of the chelates and an interaction between the metal ion and the lipid is favoured. This may lead to the breakdown of the permeability barrier of the cell ensuing in interference with the normal cell processes.

The Schiff base ligand has modest inhibitory property on the growth of the tested microorganisms. This is due to the presence of azo-methine groups which have chelating properties. These properties may be used in metal transport across the bacterial membranes or to attach to the bacterial cells at a specific site from which it can interfere with their growth. The antimicrobial activity of metal complexes shows greater bactericidal and fungicidal activities as compared to their corresponding Schiff base. The present investigations of antimicrobial activity data indicated that all of the newly synthesized complexes exhibited different antimicrobial activity as compared to that of the control drugs. According to these data, it can be easily concluded that these complexes might be recommended and/or established as new candidates for the search for new antibacterial and antifungal agents.

CONCLUSION

In the present study, four Schiff base first series transition metal complexes have been synthesized and characterized by physico-chemical and various spectral techniques. The low molar conductance value indicates that all the complexes are non-electrolytic in nature. ^{1}H NMR and FT-IR spectra revealed the tetradentate coordinating mode of the Schiff base ligand. Based on physicochemical and spectral studies an tetrahedral geometry have been assigned for Co(II), Ni(II) and Cu(II) complexes and octahedral geometry for Zn(II) complex.

The antitumour studies of the Schiff base and its Co(II), Ni(II), Cu(II) and Zn(II) metal complexes have better and promising results. *In vitro* cytotoxicity studies on VEAc and its different transition metal complexes showed cytotoxicity against DLA cell lines. The copper complex showed highest cytotoxicity with an IC_{50} value of 49 µg/ml. The present study we concluded that the copper complex of VEAc was found to be efficient against EAC-induced ascites tumour. *In vitro* cytotoxic properties of the copper complex of VEAc suggest its potential use as an anticancer agent. The synthesized ligand and the complexes were tested for their in vitro antimicrobial activity against two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. The antimicrobial activity of metal complexes shows greater bactericidal and fungicidal activities as compared to their corresponding Schiff base. According to these data, it can be concluded that these complexes might be suggested and/or established as new candidates for the search for new antibacterial and antifungal agents.

CONFLICTS OF INTEREST:

The authors declare that there is no conflict of interests regarding the publication of this paper.

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