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Mixed ligand complexes of Cu (II) / Ni (II) / Zn (II) ions with 5-Fluorouracil (5-FU) in the presence of some amino acid moieties: Structural and antimicrobial studies

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ABSTRACT

The coordinating character of 5-Fluorouracil (5-FU) (A) in the presence of some amino acid moieties (B) [B = alanine (ala) and phenylalanine (pal)] with N and O sites towards some metal ions namely Ni(II), Cu(II) and Zn(II) of MAB type mixed ligand complexes have been synthesized and characterized by elemental, spectral (vibrational, electronic, ^1H NMR and ESR) data as well as by magnetic moment values and conductivity measurements. The elemental analysis suggests that the stoichiometry of the Metal:5-FU(A):ala/pal(B) mixed ligand complexes to be 1:1:1. The low molar conductance values reveal the non-electrolytic nature of these complexes. Magnetic moment values in concert with the electronic spectra indicate that 5-FU coordinates with the metal ion in a bidentate manner through the $C_{(4)} = O$ and $N_{(3)}$ atoms also identify that the amino acids behave as bidentate by nitrogen and carboxylato oxygen. The antimicrobial activity of the synthesized complexes also is screened against bacteria and fungi. Cu(II) and Ni(II) mixed ligand complexes show an increased activity in comparison to the controls.

Keywords: Mixed ligand complexes, 5-Fluorouracil, Amino acid moieties, Spectral studies, Antimicrobial activity.

INTRODUCTION

Structural change of 5-Fluorouracil (5-FU), a mono fluorinated result of uracil, has utterly different biological properties than uracil which has substantial biological applications when it forms complexes with metal ions [1]. As the time of its synthesis, 5-FU has been ever more in work alone or in combination with other cytotoxic drugs and hormones in the medical treatment of solid tumours. It has also been used in the healing of breast, lung, ovary and cervix carcinomas [2]. This is owing to the presence of fluorine atom at the vital C-5 position which can considerably modify the electronic properties of the pyrimidines as indicated experimentally by changes in the electronic spectra [3]. Amino acids like glycine, alanine, valine, phenylalanine etc., comprise the building blocks of proteins and are chemical species necessary for performing a massive quantity of biological functions, as exemplified by the part of enzymes [4]. Mixed ligand complexes of amino acids are involved in the exchange and transport mechanism of trace metal ions in the human body [5]. Udai P. Singh et al. [6], J. Huang et al. [7] and Joshi et al. reported synthesis and characterization of transition metal complexes of Cu(II), Ni(II) and Zn(II) with 5-Fluorouracil and some amino acids. The literature reveals that number of drugs have been used to synthesize the complexes by means of many metals with an observation to improve their therapeutic action [8 -11].

In this paper, we report the synthesis, spectral and biological activities of mixed ligand Ni(II), Cu(II) and Zn(II) complexes involving 5-Fluorouracil (5-FU) and amino acid ligands viz., and alanine (ala) and phenylalanine (pal).

This study helped in understanding the coordination environment of the ligands around the metal ion and to investigate the antimicrobial activities.

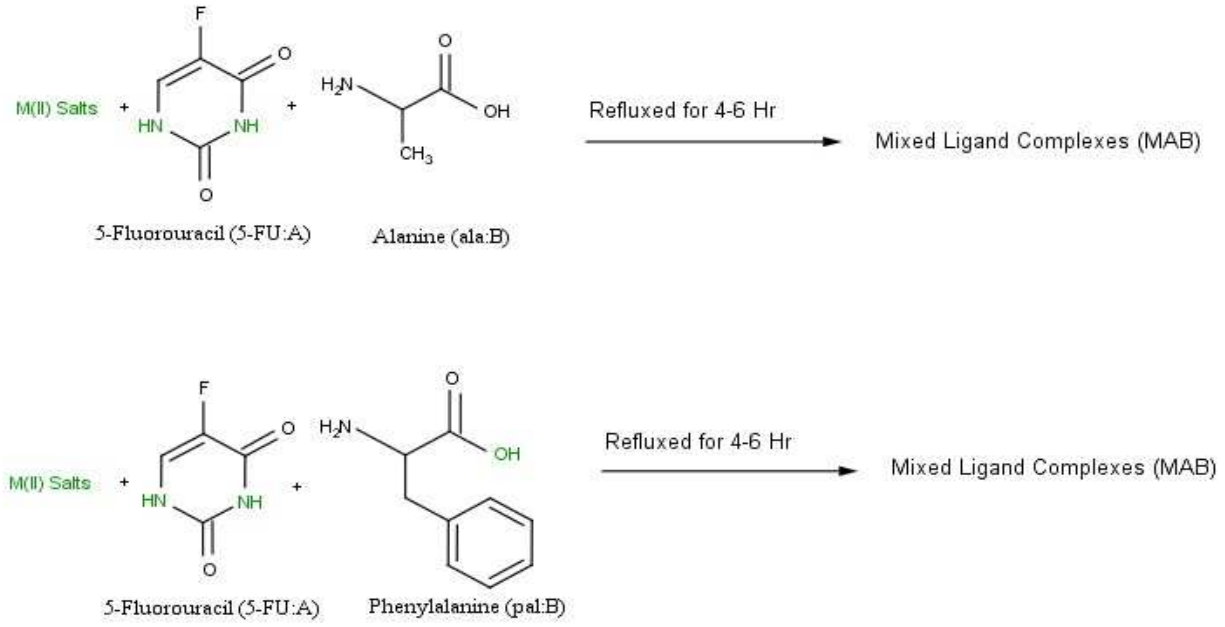
EXPERIMENTAL SECTION

All the ligands are extra pure Sigma Aldrich, Fluka (Puriss) products and they are used without further purification. Solvents for the physical measurements were of analytical grade and purified according to literature methods [12]. Melting point (mp) of all the mixed ligand complexes was determined on Gallenkamp apparatus in open glass capillaries and is uncorrected. C, H and N analytical data were performed on Elementar Vario EL III CHNS analyzer. Metal content of the mixed ligand complexes were estimated gravimetrically by the standard procedure. Molar conductance (1×10^{-3} M) was measured using an Elico CM 180 conductivity bridge by using 0.01 M KCl solution as calibrant. Magnetic susceptibility measurements were carried out on a Gouy balance at room temperature using mercuric tetra(thiocyanato)cobaltate(II) as the calibrant. Diamagnetic corrections were applied in compliance with Pascal's constant [13]. Electronic absorption spectra were recorded with a Hitachi U–2000 double beam spectrophotometer in the 200–1100 nm range. Vibrational spectra were recorded using KBr pellets on a JASCO FT/IR–410 spectrometer, in the 400–4000 cm^{-1} range. ^1H NMR spectra of the diamagnetic Zn(II) complexes were carried out in DMSO–d_6 at room temperature using TMS as internal standard on a Perkin Elmer R–32 spectrometer. X-band ESR spectra of Cu(II) complexes at room temperature and liquid nitrogen conditions in DMSO medium were recorded on a Varian ESR spectrometer using DPPH as internal standard. *In vitro* antimicrobial activities of 5-Fluorouracil(A) and their Ni(II)/Cu(II)/Zn(II)–5–FU(A)–ala/pal(B) complexes in DMSO medium were tested against three Gram–positive pathogenic bacterial strains: *Bacillus subtilis*, *Staphylococcus saphyphiticus* and *Staphylococcus aureus*, two Gram–negative bacterial strains: *Escherichia coli* and *Pseudomonas aeruginosa* using Muller Hinton agar nutrient and three fungal strains namely *Aspergillus niger*, *Enterobacter species* and *Candida albicans* using potato dextrose agar medium by well diffusion technique [14].

Synthesis of mixed ligand complexes

5–Fluorouracil (0.013 g, 10 mmol) was dissolved in aqueous (10 ml) solution containing a few drops of concentrated ammonia and stirred. When 5-fluorouracil was completely dissolved, an aqueous (10 ml) solution of appropriate metal salt (10 mmol, 0.025 g $Ni(CH_3COO)_2 \cdot 4\ H_2O$ or 0.020 g $Cu(CH_3COO)_2 \cdot H_2O$ or 0.022 g $Zn(CH_3COO)_2 \cdot 2\ H_2O$) solution was added slowly and stirred at room temperature (**Scheme 1**). To this solution, aqueous solution (10 ml) of alanine (0.009 g, 10 mmol) or phenylalanine (0.017 g, 10 mmol) was added and the reaction mixture was refluxed for 4–6 h on a water bath. The pH (= 6.3) of the reaction mixture, adjusted by adding few drops of aqueous Na_2CO_3 solution (0.0105 g, 10 mmol). The resulting solution were reduced to 1/3 of its original volume by water bath and kept aside. On standing, the mixed ligand complexes were obtained and collected by *vacuum* filtration, washed several times with cold water, ethanol and anhydrous ether. The mixed ligand complexes were dried in air and stored in *vacuo* over anhydrous $CaCl_2$ at room temperature. The yield of the isolated complexes was found to be 60–70%.

Scheme - 1. Formation of mixed ligand complexes (MAB)



RESULTS AND DISCUSSION

The elemental analysis and Molar conductance

The analytical data show, the stoichiometry of M(II) : 5-Fluorouracil : alanine/ phenylalanine is to be 1:1:1 for these MAB type mixed ligand complexes and the values are in good concord with the calculated values (Table 1). The observed low molar conductance values (1×10^{-3} M, DMSO solution) at room temperature are reliable with the non-electrolytic nature due to the absence of counter ions in the proposed structures [15].

Table 1. Physico-chemical properties of mixed ligand complexes

Complex	Colour	Empirical Formula	Yield (%)	Elemental analysis, found (calculated) %				Λ_m (Ω^{-1} cm^2 mol^{-1})	M.pt (^oC)
				M	C	H	N		
(1)Ni(II)-5-FU-ala	Greenish Blue	$NiC_9H_{14}FO_7N_3$	63	16.61 (16.64)	30.59 (30.62)	3.95 (3.97)	11.86 (11.91)	11.1	227
(2)Cu(II)-5-FU-ala	Bluish Green	$CuC_7H_9FO_4N_3$	61	22.58 (22.61)	29.84 (29.89)	3.18 (3.21)	14.88 (14.95)	14.8	233
(3)Zn(II)-5-FU-ala	Colourless	$ZnC_9H_{14}FO_7N_3$	66	18.17 (18.19)	30.10 (30.05)	3.91 (3.95)	11.54 (11.69)	14.1	226
(4)Ni(II)-5-FU-pal	Pale Green	$NiC_{15}H_{18}FO_7N_3$	60	13.58 (13.69)	41.94 (41.99)	4.19 (4.26)	09.31 (09.69)	10.9	242
(5)Cu(II)-5-FU-pal	Deep Blue	$Cu\ C_{13}H_{13}FO_4N_3$	63	17.73 (17.80)	43.63 (43.69)	3.61 (3.64)	11.87 (11.77)	10.2	251
(6)Zn(II)-5-FU-pal	Colourless	$ZnC_{15}H_{18}FO_7N_3$	61	14.30 (14.41)	39.63 (39.68)	3.88 (3.97)	09.17 (09.23)	11.7	261

Vibrational spectra

The IR spectra provide valuable information on the subject of the nature of functional group attached to the metal ion. The characteristic IR spectral data (KBr pellet, cm^{-1}) with the principal IR frequencies of 5-FU and its mixed ligand complexes are given in (Table 2). The presence of coordinated water molecules ν(OH) is confirmed by the rocking, twisting and wagging vibrational modes at 3101 – 2931 cm^{-1} , 954 – 949 cm^{-1} and 747 – 739 cm^{-1} respectively [16] except for Cu(II) complexes. ν(M–N) and ν(M–O) bands are tentatively assigned in the region 439–432 cm^{-1} and 551–542 cm^{-1}indicating the complexation of the ligands with transition metal ions [17] respectively.

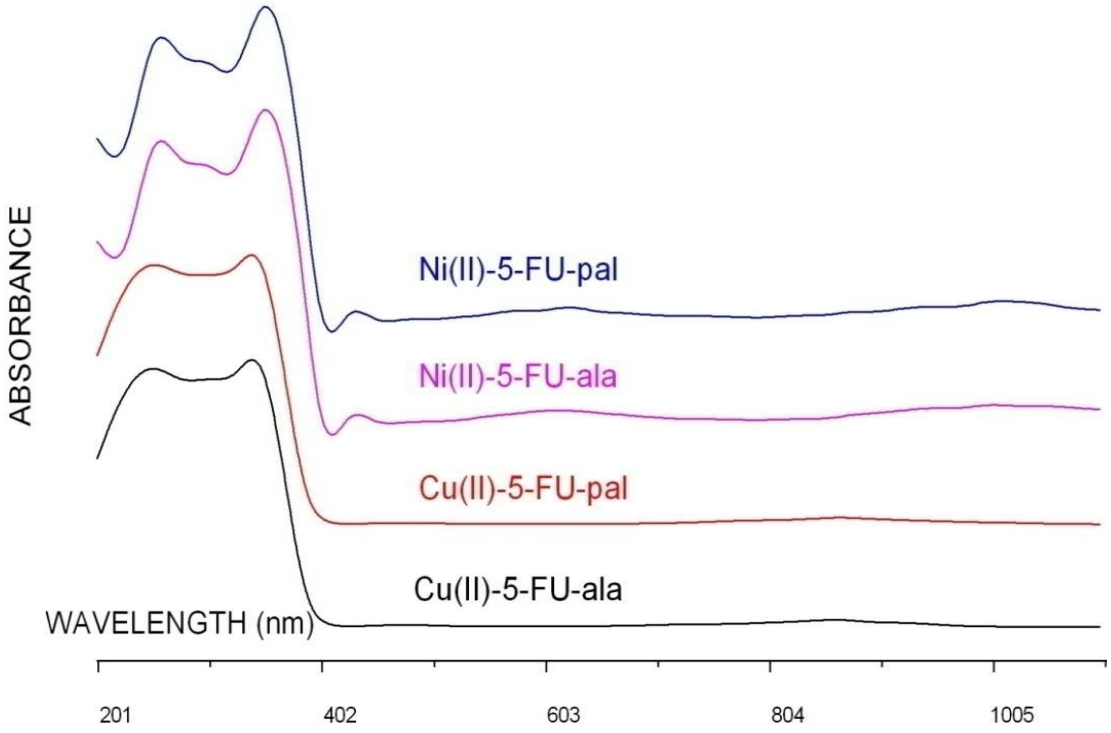
Table - 2. IR spectral data (in cm^{-1}) of mixed ligand complexes

Complexes	υ (OH_2)	υ (C=O) of C_2 & C_4 pyrimidine ring	δ(N-H) of N_1 & N_3 Pyrimidine ring	υ (C_5-F) pyrimidine ring	$\upsilon(COO)_a$	$\upsilon(COO)_s$	υ(M-N)	υ(M-O)
(1)5-Fluorouracil	-	1704, 1685	1513, 1430	1470	-	-	-	-
(2)Ni(II)-5-FU-ala	3227-2933	1691, 1652	1517,1408	1473	1664	1412	432	542
(3)Cu(II)-5-FU-ala	954, 740 -	1697, 1662	1511, 1408	1471	1666	1415	432	543
(4)Zn(II)-5-FU-ala	3329-2935	1703, 1661	1519,1408	1468	1612	1362	434	545
(5)Ni(II)-5-FU-pal	949, 738 3111-2933	1708, 1664	1516,1417	1470	1650	1413	430	546
(6)Cu(II)-5-FU-pal	950, 740 -	1701, 1677	1517,1414	1467	1654	1410	433	551
(7)Zn(II)-5-FU-pal	3101-2931	1699, 1661	1511,1410	1472	1657	1414	439	547
	953, 747							

From the data, the ligand 5–FU(A) acts as bidentate which form metal chelates all the way through the deprotonated N_3 and C_4 = O of carbonyl oxygen atoms. Likewise, the ligands alanine/phenylalanine (B) binds the M(II) ions in bidentate manner through amino –N and deprotonated carboxylato –O atoms which forms a stable 5, 6 membered chelation. The disappearance of amino $–NH_2$ band (3340cm^{-1}) of free amino acid ligands indicating the participation of this group in chelation. All these complexes show an additional characteristic bands in the region 1666–1612 cm^{-1} and 1415–1362 cm^{-1} are ascribed to ν(C–O) of asymmetric and symmetric vibration of the carboxylate (COO^-) group respectively [16], indicating the participation of the carboxylate oxygen in the mixed ligand complex

formations. The magnitude of $\Delta\nu$ value falls in the range 237–252 cm^{-1} suggesting the unidentate coordination of in complexation and forming N_2O_2 type of environment around the central metal ion.

Figure – 1 Electronic absorption spectra of Ni(II)- 5-FU(A)-ala/pal(B) and Cu(II)-5-FU(A)- ala/pal(B) systems.



Electronic absorption spectra coupled with Magnetic moment values

The electronic absorption spectra of complexes were recorded in (1×10^{-3}M) DMSO medium at room temperature (Figure 1). Also, various spectral parameters (B, B^0, β, β^0 (%)and LFSE) for Ni(II) complexes were calculated by applying band energies on Tanabe Sugano diagrams and the data are given in Table 3. These values are helpful to identify their coordination environment [18, 19].

The spectra suggested distorted tetrahedral, octahedral, distorted octahedral for Cu(II), Ni(II) and Zn(II) mixed ligand complexes respectively. The magnetic moment values of these mixed ligand complexes in Bohr Magneton support their proposed geometry [20].

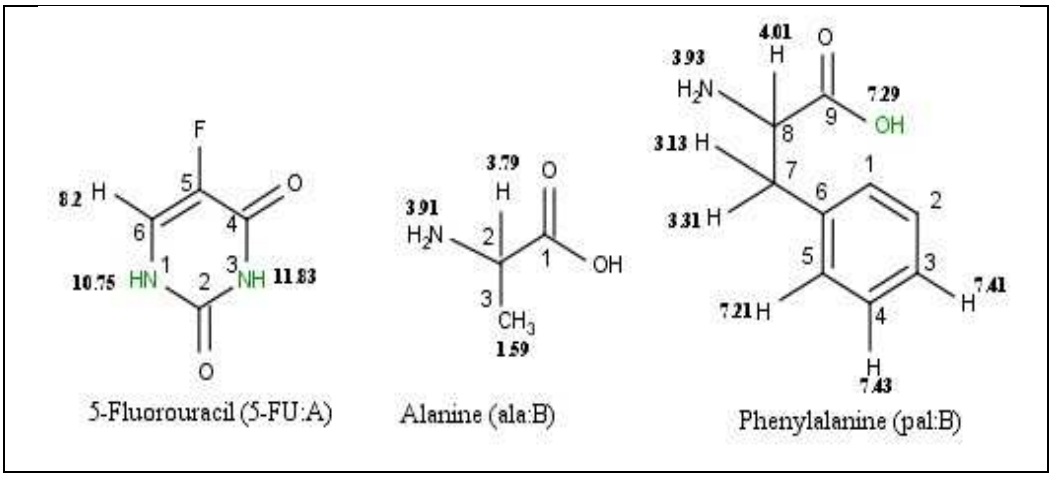
^{1}HNMR spectra of zinc complexes

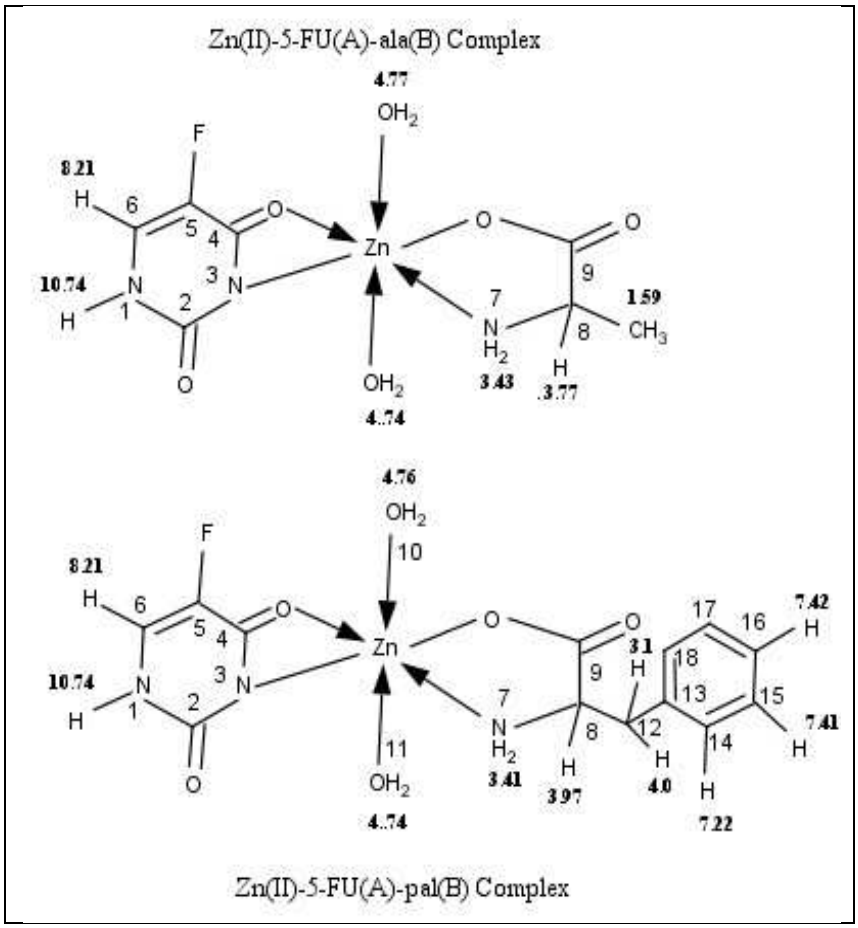
The ^{1}H NMR spectra of diamagnetic Zn(II)-5-FU-ala/pal complexes were recorded in d_6-dimethylsulfoxide (DMSO) solution using tetramethylsilane (TMS) as internal standard at room temperature. The representative structure of the Zn(II)-5-FU-ala/pal (B) complexes were compared with the free ligands, 5-FU(A) and ala/pal(B) which are shown in Figure 2. From the NMR spectra, 5-FU(A) and ala/pal(B) ligands act as bidentate *via*, deprotonated N_3, carbonyl oxygen (C_4 = O) and amino –N, deprotonated carboxylate –O atoms respectively. Also, there is a peak found at 4.7 ppm in the Zn(II) complexes indicating the presence of coordinated water molecules in the complexes [21, 22]. Thus, the NMR study reinforces the conclusion drawn from the IR spectra.

Table - 3. Absorption spectral data (in DMSO) and magnetic susceptibility of 5-FU Mixed Ligand complexes MAB at 37 °C

Complex	λ_{max} (cm^{-1})	Band assignments	Geometry	μ_{eff} (BM)	Ligand field parameter					
					Dq (cm^{-1})	B (cm^{-1})	β'	β^0 (%)	LFSE (kJ mol^{-1})	ν_2/ν_1
Ni(II)-5-FU-ala	10,066 15,872 26,181	$^3A_{2g}$ (F) → $^3T_{2g}$ (F) $^3A_{2g}$ (F) → $^3T_{1g}$ (F) $^3A_{2g}$ (F) → $^3T_{1g}$ (P)	Octahedral	3.02	1,007	790 (1030 for free ion)	0.76	24.88	120.7	1.57
Cu(II)-5-FU-ala	11,587	d-d envelope	Distorted tetrahedral	1.97	--	--	--	--	--	--
Zn(II)-5-FU-ala	26,576 (b)	LMCT (M ← N)	Distorted octahedral	Dia.	--	--	--	--	--	--
Ni(II)-5-FU-pal	10,068 15,873 26,181	$^3A_{2g}$ (F) → $^3T_{2g}$ (F) $^3A_{2g}$ (F) → $^3T_{1g}$ (F) $^3A_{2g}$ (F) → $^3T_{1g}$ (P)	Octahedral	2.98	1,007	776 (1030 for free ion)	0.75	25.11	120.8	1.58
Cu(II)-5-FU-pal	11,614	d-d envelope	Distorted tetrahedral	1.98	--	--	--	--	--	--
Zn(II)-5-FU-pal	27,624 (b)	LMCT (M ← N)	Distorted octahedral	Dia.	--	--	--	--	--	--

Figure - 2. ^{1}H NMR of the free ligands (A & B) and their Zn(II)-5-FU(A)-alanine/phenylalanine (B) complexes





ESR spectra

The X-band ESR spectra of Cu(II)-5-FU-ala/pal complexes in DMSO were recorded at ambient and low (77 K) temperatures. At 300 K, ESR spectra display well resolved hyperfine structure because of magnetic coupling between the unpaired electron by effective ^{63,65}Cu nuclei (I = 3/2). The hyperfine lines vary in intensity owing to the tumbling motion of the molecules.

The observed $A_{||}$ =128 x 10^{-4} cm and 126 x 10^{-4} cm for Cu(II)-5-FU-ala/pal complexes respectively are comparable to the reported distorted tetrahedral Cu(II) complexes [23]. Also the ratio of $g_{||}$ / $A_{||}$ value are calculated as above 172 cm, which is comparable to a distorted tetrahedral environment around the Cu(II) ion. These reduced $A_{||}$ values in tetrahedral symmetry are explained by the fact that the 4s and 4p orbitals in the ground state can be easily mixed in low symmetry complexes [24].

Table - 4 The Spin Hamiltonian parameters of complexes in DMSO at 300 and 77 K

Complex	Hyperfine constant X 10^{-4} cm^{-1}										
	$A_{\|\|}$	$A_{\perp}$	A_{iso}	$g_{\|\|}$	$g_{\perp}$	g_{iso}	α^2	β^2	α	$g_{\|\|}/A_{\|\|}$	G
Cu(II)-5-FU-ala	128	43	72	2.22	2.01	2.11	0.62	0.84	0.79	173	28.28
Cu(II)-5-FU-pal	126	40	70	2.22	2.01	2.10	0.61	0.83	0.78	176	28.27

The calculated value of Fermi constant, κ = 0.371 by the expression [25] 20],

$P = 2\,\gamma_{Cu}\,\beta_o\,\beta_N\,(r^{-3}) = 0.0363\ cm^{-1}$ $\qquad \kappa = \left(\frac{A_0}{P}\right) + \Delta g_0$

This value is similar to the general order [26]. The absence of any half field signal at 1600 G corresponding to Δ Ms = ± 2 transitions, ruling out any magnetic exchange i.e., absence of Cu–Cu interactions in the complexes. Hathaway [27] pointed out that for the pure σ-bonding $K_{\|} > K_{\perp} \approx 0.767$ and for in-plane π-bonding $K_{\|} < K_{\perp}$, while for out-of-plane π-bonding $K_{\|} > K_{\perp}$ the following simplified expressions were used to calculate $K_{\|}$ and $K_{\perp}$.

$$K_{\|} = \left(\frac{g_{\|} - 2.0023}{8\,\lambda_0}\right) \text{x d – d transition}$$

$$K_{\perp} = \left(\frac{g_{\perp} - 2.0023}{2\,\lambda_0}\right) \text{x d – d transition}$$

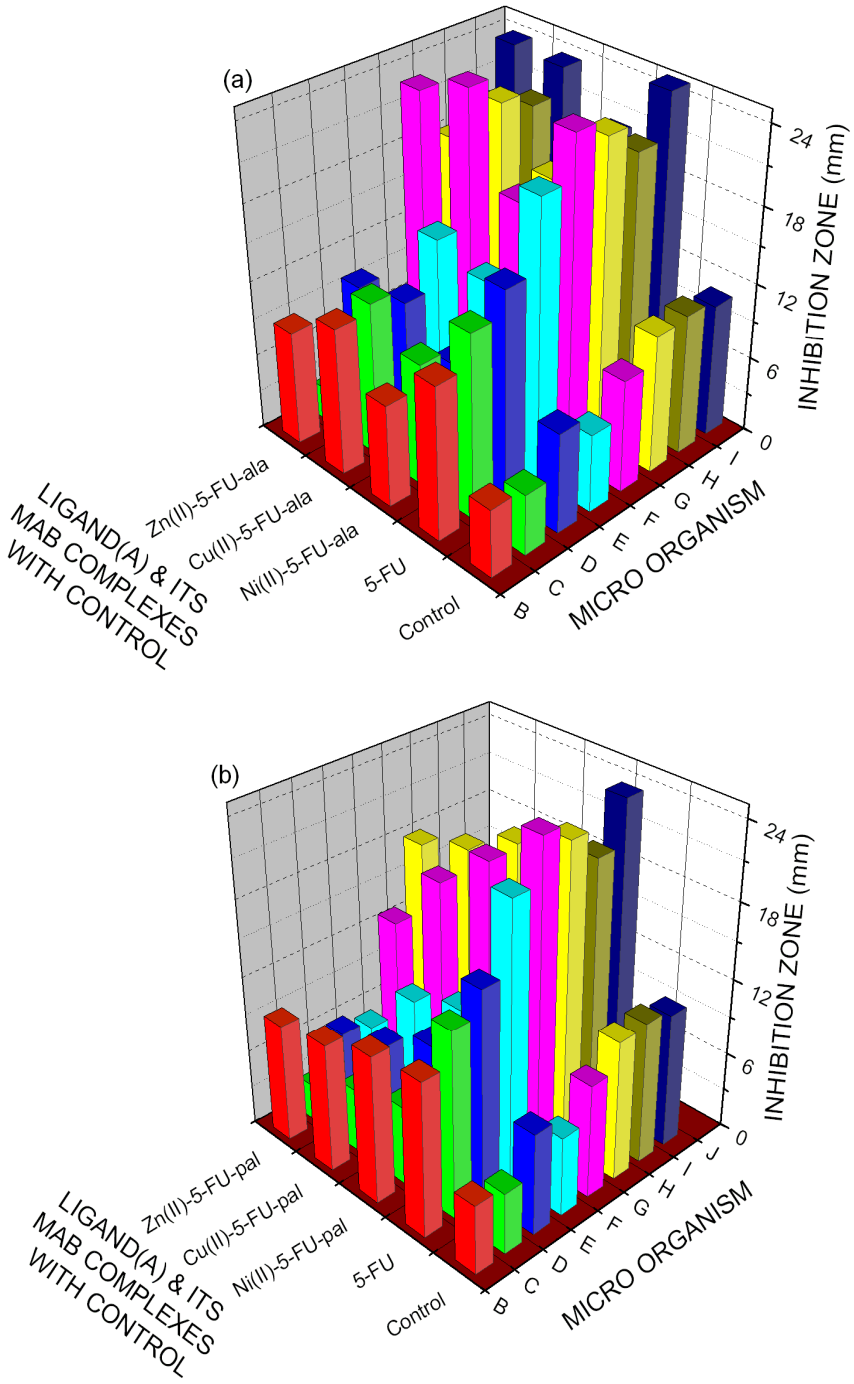
The observed $K_{\|} > K_{\perp}$ relation indicates the absence of significant in-plane π-bonding. Additionally, the estimated α^2 and β^2 values obtained by Kivelson and Neimann formula, reflects the high covalency of the Ligand-Cu bonding. The degree of distortion is given in Table 4. The distortion from the plane increases with increasing $g_{\|}$ values and decreasing $A_{\|}$ values.

Table - 5.Antimicrobial activity of the free ligand(A) and their mixed ligand complexes (Minimum Inhibitory Concentration values at 1×10^{-2} M).

[a] Average of three replicates.

Complex	Minimum Inhibitory Concentration values (µg / ml) [a]							
	Bacterial strains					Fungal strains		
	Bacillus subtilis	*Staphylococcus saphyphiticus*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Enterobacter species*	*Candida albicans*
Control	5.4	4.8	7.9	6.2	8.9	11.0	11.2	10.6
5-FU(A)	12.1	14.6	16.4	21.8	25.1	23.8	21.6	24.9
Ni(II)-5-FU-ala	8.1	9.8	8.3	13.5	18.3	18.9	13.8	20.0
Cu(II)-5-FU-ala	11.7	12.3	11.0	14.7	24.9	22.8	21.6	23.5
Zn(II)-5-FU-ala	9.1	2.7	10.1	5.4	23.1	18.4	20.1	23.7
Ni(II)-5-FU-pal	11.6	6.2	9.8	11.4	21.5	21.8	18.2	16.8
Cu(II)-5-FU-pal	10.1	5.0	7.5	9.9	18.1	19.6	17.2	15.9
Zn(II)-5-FU-pal	9.3	2.9	6.2	5.5	13.0	18.2	10.3	13.3

Figure - 3. Biological activities of (a) Ligand (A) & M(II)-5-FU(A)-ala(B) complexes with control and (b) Ligand(A), M(II)-5-FU(A)-pal (B) complexes with control in different microorganism species at 24 h respectively.



B = *Bacillus subtilis* C = *Staphylococcus saphyphiticus* D = *Staphylococcus aureus* E = *Escherichia coli* F = *Pseudomonas aeruginosa* G = *Aspergillus niger* H = *Enterobacter species* I = *Candida albicans*

Antimicrobial activity

In vitro minimum inhibitory concentration (MIC) values of the mixed ligand complexes of Ni(II) / Cu(II) / Zn(II)–5–FU(A)–ala/pal(B) complexes with free ligand (5–FU) were tested against some microorganisms and are summarized in Table 5. Commercially existing standard drugs Ampicillin (antibacterial control) and Nystain (antifungal control) are used as control. The antimicrobial activities of the mixed ligand complexes are normally better than the free ligands and the activities depend upon the M(II) ions i.e., size, charge distribution, shape and redox potential of the metal chelates [28]. The mixed ligand complexes explain moderate activity than the corresponding free ligands and metal salts. From the Table 5, the MIC value of all the mixed ligand complexes were also confirmed by the liquid dilution route [29] in which the efficacy was also observed at very low concentrations.

The representative graph of the measured MIC values (μg / ml) of the controls, mixed ligand complexes with free ligands against microorganisms are shown in Figure 3 which can be explained on the basis of chelation theory [30]. From the MIC values, Ni(II)/Cu(II)–5–FU(A)–ala/pal(B) complexes show moderate activity against all the bacterial and fungal strains even than the standard drugs. Also the antibacterial activity of mixed ligand complexes is due to the presence of electron withdrawing (C_5–F) and amino acids, alanine(B) and phenylalanine(B) moieties in their nuclei. Also, it was established that the pathogenic Gram positive bacterial strains like *Bacillus subtilis* further, *Escherichia coli* and fungal strains like *Aspergillus niger and Enterobacter species* show remarkable activities against the free ligands and their mixed ligand complexes.

CONCLUSION

The present study explains geometry and nature of the mixed ligand complexes which were also studied by the electronic absorption spectra. From the spectral studies, the ligand 5–FU(A) and ala/pal(B) bind the M(II) ion via, deprotonated N_3, C_4 = O of carbonyl oxygen atoms and amino –N and also by deprotonated carboxylato –O atoms respectively, forming a stable 4, 5, 6 membered chelate rings. All the Cu(II) mixed ligand complexes under investigation show distorted tetrahedral geometry which is further supported by the ESR studies. The *in vitro* antimicrobial evaluations show more potent activities for both Ni(II) and Cu(II) mixed ligand complexes.

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