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RESEARCH ARTICLE

Synthesis, Characterization of Mixed Cu(II) Pyridyl Tetrazoles and 1,10-Phenanthroline Complexes - DFT and Biological Activity

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Abstract:

Background:

Mixed ligand copper complexes with 1,10-phenanthroline show good chemical nuclease activity and anticancer activity. Recently, tetrazole derivatives are also promising candidates for anticancer activity. Hence, it is significant to study the DNA binding and anticancer activity of two active N-donor ligands and their copper complexes.

Objectives:

The main objective of this study was to investigate the regioisomeric mixed ligand copper complexes response with calf thymus DNA binding and anti-toxic activity against MCF-7 cell line.

Methods:

The DNA binding interactions of complexes **1-4** with calf thymus DNA (CT-DNA) were monitored by UV/VIS spectroscopy. The absorption spectra of the Cu complexes are compared with and without CT-DNA at 400-450 nm. The cell proliferation was measured by using the standard 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium- 5-carboxanilide (XTT) assay with four different concentrations of the compounds (5, 10, 50, and 100 mm) and cisplatin (as a positive control) was tested in triplicate for 48 h. The results obtained by the XTTassay are expressed as the average standard deviation of two experiments. The IC_{50} values of the complexes exhibited differential and dose-dependent inhibitory activities on the growth of MCF-7 cancer cells.

Results:

Based on the elemental analysis, molar conductance, magnetic moments, mass, electronic, ESR and IR spectral data, the copper is coordinated by N-atoms of 1,10- phenanthroline and pyridyl tetrazole with octahedral structure. DFT calculations of HOMO and LUMO studies showed that electron density is localized on pyridyl tetrazole ring and phenanthroline ring. The calculated DNA binding constant (K_b) values of **1-4** complexes are in the range 4.2 - 7.6 $x10^4 M^{-1}$ (Table **4**) with similar binding affinity to reported copper tetrazole derivative complexes. The **1-4** complexes with CT DNA interaction are through planar phenanthroline and pyridyl tetrazole ring likely *via* π-stacking interactions. The IC_{50} values of complexes show excellent activity with 24(± 0.5); 18(± 0.5); 20(±0.5); (±0.5) and 38 (±0.8) for **1, 2, 3, 4** and *cis* platin complexes, respectively. After 72 h of the treatment of **1** on MCF-7 cell, IC_{50} values hinder the cell growth upto 24(± 0.5) µg/ml at 5 µM concentration range (Fig. **5**). It is apparent from IC_{50} values that the order inhibition is **1** > **3** >**2** > **4**.

Conclusion:

Experimental results are highly encouraging to explore the mixed ligand regio isomeric copper complexes which have shown the parallel result with Cisplatin. By proper structural modification of pyridyl tetrazole ligand, substituent better anticancer agents can be prepared.

Keywords: 1,10-phenanthroline, Tetrazole, DNA binding, Anticancer, XTTassay, MCF-7.

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1. INTRODUCTION

A large number of potential ligands compete for metal ions in biofluids [1]. The storage and transport of active substances

through bio-membranes is related to the specific structure of the complexes [2]. N-donar ligands are biologically important, have a key role in coordinating copper metal and their application as anticancer agents [3, 4]. Tetrazoles are an important class of N-donor ligands that are metabolically stable substituent for -COOH functional groups; assist in coordination chemistry as ligands, lipophilic spacers [5]. Introducing tetrazole ring into the molecule will reduce the toxic properties of a drug, because it exhibits stronger resistance to *in vivo* metabolization than the carboxylate group, causing the drug to stay longer (bioavailability) in blood [6, 7]. A large number of pharmacological applications of tetrazoles with anticonvulsant, antihypertensive, anti-inflammatory, antibacterial, antifungal, anticancer, glycosidase inhibitory, antidiabetic, antiulcer and antitubercular activities are reported [6 - 9]. Recently, Yang *et al*. and others invented tetrazole derivatives as promising candidates for anticancer activity [10, 11]. Anticancer and DNA binding studies of tetrazole complexes are studied rarely [12, 13].

While N-donar ligand, such as 1,10-phenanthroline, their copper complexes have been reported as anticancer agents since the initial discovery of the nuclease activity of $Cu(phen)_2$ by Sigman and co-workers. Their action relies mainly on oxygen activation and DNA oxidation [14, 15]. Mixed ligand copper complexes with 1,10-phenanthroline have shown good chemical nuclease activity, preferably in the presence of molecular oxygen and a reducing agent on double-stranded DNA [16, 17]. In continuation of our search for better chemical nucleases [18], newly mixed ligand copper complexes with phenanthroline and pyridyl tetrazoles groups investigated their DNA binding studies and anticancer activity on MCF cell lines.

2. MATERIALS AND METHODS

All chemicals were purchased from Sigma–Aldrich of reagent grade and distilled solvents were used in the synthesis of the ligands and metal complexes. Agarose, calf thymus DNA and plasmid pBR322 were purchased from Genie Biolabs, Bangalore, India. The elemental analyses of complexes were performed by Perkin Elmer CHNS analyzer. Molar conductance was measured using a Digisun conductivity metre in DMF solvent. Mass spectral data is acquired by Q1MSQ1/ auto-injection method on PeSciex API 2000 eV spectrometer. Mass spectra were obtained on a PeSciex API 2000 eV spectrometer and Q1MSQ1/auto-injection mass spectra IR spectra were recorded on Alpha T OPUS spectrometer instrument. PAR model-155 vibrating sample magnetometer is used for magnetic moments studies in the polycrystalline state at a field strength of 2-8 kg with Ni metal as standard. EPR spectra were recorded on a Varian E-122 X-band spectrometer at liquid nitrogen temperature in DMF. TGA/DTA measurements were performed TA Instruments, Model SDT 2960 analyzer with a heating rate of $5^oC\ min^{-1}$ in range of 25–500°C, under nitrogen flow on about 10 mg of sample in an aluminum crucible. DNA binding studies and cytotoxic studies were studied as per literature and our previous paper [18, 19]

2.1. Synthesis of Complexes

The appropriate ligands (**L1-L4**) (1.60 mmol) [18] were dissolved in ethanol (25 ml) and added to a $CuCl_2.H_2O$ (1.36 mmol) and phenanthroline(1.36 mmol) in ethanol solution (10 ml) at 120°C in Teflon lined stainless steel reactor for 48hrs by solvothermal method. The resulting green colored powder is filtered, dried and collected.

[Cu(**L1**)(Phen)]Cl_2(**1**): Green powder - (24 mg, yield 38%). Calcd. for $C_{22}H_{22}CuN_8Cl_2$, Mol. Wt. (532): Carbon, 49.58%; Hydrogen, 4.16%; Nitrogen, 21.03% ; Found: Carbon, 49.18%; Hydrogen, 4.04%; Nitrogen, 21.04%.

[Cu(**L3**)(Phen)]Cl_2 (**3**): Green solid (21 mg, yield 34%). Calcd. for $C_{20}H_{17}CuN_7OCl_2$; Mol. Wt.: 491; Carbon, 46.40% ; Hydrogen, 3.07%; Nitrogen, 19.94; Found: Carbon, 46.13% ; Hydrogen, 3.02%; Nitrogen, 19.86%.

3. RESULTS AND DISCUSSION

The regioisomers 2-[5-(pyridin-2-yl)-1H-tetrazole]propyl-N,N-dimethylamine] **L1**, (2-[5-(pyridin-2-yl)-2H-tetrazol-2-yl] propyl-N,N-dimethylamine) **L2** and **L3** (2-(1-ethanol-1H-tetrazol-5-yl)-pyridine); **L4** (2-(2-ethanol-2H-tetrazol-5-yl)pyridine) were prepared as per our previous paper [18]. A clear ethanolic solution of pyridyltetrazole derivatives (**L1–L4),** 1,10 - phenanthroline and $CuCl_2.2H_2O$ in 1:1:1 ratio was heated at 120°C in Teflon line steel reactor to give [Cu(L)(phen)] complexes. These complexes are soluble in DMF, DMSO solvent and slightly in ethanol. The molar conductivity of complexes is shown in Table **1** (54.8 - $86.4\Omega^{-1}cm^2mol^{-1}$), suggesting that complexes are highly stable in solution. The magnetic moment values of **1-4** complexes are more than the 1.73 μ (spin-only values), expected for a d9 copper (II) system [20].

Table 1. Physical date of copper complexes (1- 4).

S.No	Copper Complexes	Colour	Melting Point °C	Λ_m/Ω^{-1} cm^2 mol^{-1}	μeff (BM)
1	[Cu(**L1**)(Phen)]Cl_2	Green	258-260	86.4	1.92
2	[Cu(L2)(Phen)]Cl_2	Green	248-250	65.8	1.84
3	[Cu(**L3**)(Phen)]Cl_2	Green	235-238	74.6	2.04
4	[Cu(**L4**)(Phen)]Cl_2	Green	224-226	54.8	1.89

The Electronic molar extinction coefficient data of the copper complexes (**1-4**) are shown in Table **2**. These complexes show charge transfer band in 27,450-33,560-cm^{-1} region. While single broadband observed due to the $^2E_g\ ^2T_{2g}$ transition in the region 8540- 11580 cm^{-1} is assigned to d-d transition. These transition bands strongly favor a distorted octahedral geometry around the metal ion, which is supported by magnetic susceptibility values [21].

I.R. spectra of **L1** ligand show peaks at 2955, 1592 and 1450 cm^{-1} and **L3** ligand at 2885, 1575 and 1415 cm^{-1} for methylene and tetrazole ring, these peaks are shifted to a lower frequency in copper complexes, suggesting the tetrazole group with copper coordination. Additional bands are observed in IR spectra of complexes at 260–220, 245–230 cm^{-1}, apparently due to coordination with metal *via* nitrogen donor atoms.

The stoichiometry compositions of complexes are established by the FAB mass spectra. The molecular ion peak of

complexes **1** were observed at m/z = 534.9 and **2** at m/z = 504 with [Cu(**L**)(Phen)]Cl_2. The ESI-mass studies and elemental analyses are close agreement with the values calculate from molecular formula assigned to these complexes, which is further supported by of representative complexes.

Table 2. Electronic spectral data $cm^{-1}(\varepsilon_{max}$ $(M^{-1}Cm^{-1}))$ of complexes

Complexes	MLCT	d-d (Ɛ)
1	33560	11580 (68)
2	28622	9560 (46)
3	30286	10516 (52)
4	27450	8540 (42)

EPR spectra of **1** and **3** were recorded in the X-band region at room temperature in the solid state. The $g_{\parallel}$ and $g_{\perp}$ values of **1**, **3** complexes are 2.264, 2.259 and 2.058, 2.060, respectively. From Table **3**, it observed $g_{\parallel} > g_{\perp} > 2.0023$, suggesting unpaired electron lies predominantly in the $d_{X^2-Y^2}$ orbital.

Table 3. ESR spectral assignments for complexes 1 and 3 at room temperature.

Complexes	$g_{\parallel}$	$g_{\perp}$	g_{av}	G
scope="row">**1**	2.264>	2.058>	2.16>	4.69>
scope="row">**2**	2.259	2.060	2.15	4.44

The axial symmetry parameter (G) values are calculated [22, 23], the values of **1** and **3** complexes are >4 (G = 4.3), suggesting that the local tetragonal axes are aligned in parallel or slightly misaligned, unpaired electron in $d_{X^2-Y^2}$ orbital and exchange coupling effects are not operative in these complexes. The shapes of the spectra are consistent with the octahedral geometry around the Cu(II) center in the complexes [24].

Based on the elemental analysis, molar conductance, magnetic moments, mass, electronic, ESR and IR spectral data tentatively structures of **1-4** complexes are shown in Fig. (**1**).

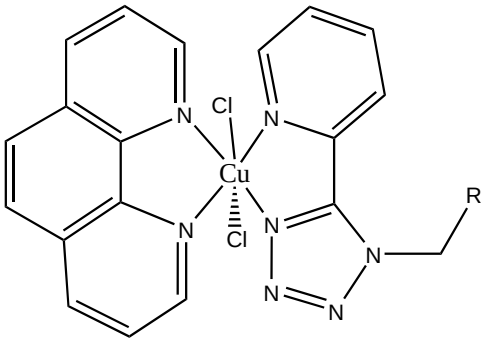
3.1. Computational Studies

Density Functional Theory (DFT) calculations at B3LYP level were carried out for geometry optimization of **1** and **3** complexes in their ground state. The geometrical optimized structure and frontier orbital's of HOMO and LUMO of **1** are shown in Fig (**2**). The measured bond lengths of Cu-N atoms of phenanthroline are 1.92°A and 1.89°A and Cu-N of pyridyl tetrazole are 1.89°A and 1.87°A, respectively.

It is clear from Fig. (**3**) that the electron density of HOMO is localized on pyridyl tetrazole ring, while electron density in LUMO is largely on phenanthroline ring. The energy gap between HOMO and LUMO of **1** and **3** complexes is -0.16594 eV and -0.05903 eV which are not influenced by excitation. The electronic density of HOMO and LUMO in singlet state suggests that the absorption transition is due to the Intra-Ligand Charge Transfer (ILCT or $\pi \rightarrow \pi^*$) and partially due to ligand-to-metal charger transfer, metal-to-ligand charger transfer and Metal Centered (MC) transitions which are supported by UV-V absorption spectra.

3.2. Thermal Studies

TGA and DTA analyses were carried out to investigate the thermal stability of the **1** and **3** complexes in static air at a temperature range 30 to 630°C with a heating rate of 10°C min^{-1}. TGA and DTA curves show a stable with no weight loss up to 130°C temperature for complex 1 (Fig. **4**). The initial degradation is due to the loss of phenanthroline and -N-$(CH_2)_2$(OH) moieties in complex (**1**), -N-$(CH_2)_3(NH_2)$ in complex (**3**) with a practical weight loss of 32.77% (Calc.31.92%) and 33.27% (Calc.31.42%). The exothermic peak of DTA curve exhibits at 171.9 and 194.0°C for **1** and **3,** respectively. Further degradation in complex **1** at 365°C and 500°C is due to loss of tetrazole species and other organic moieties with a weight loss of 48.93% (Calc. 48.37%). Degradation in complex **3** at 330°C and 530°C is by the loss of tetrazole and organic moieties with a weight loss of 47.7% (Calc. 47.56%) with final residual weight to cupric oxide.



where R = OH, $N(CH_3)_2$

Fig. (1). Tentative assigned structure for copper complexes.

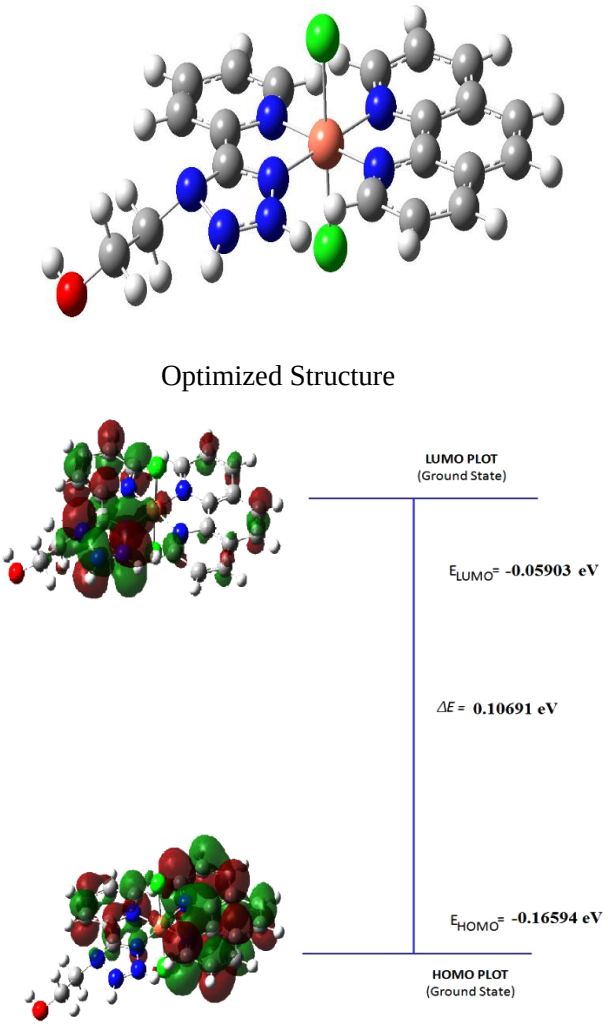


Fig. (2). Optimized structure, HOMO and LUMO orbital of complex **1**.

3.3. DNA Binding Studies

Complexes *Vs* CT-DNA binding studies were investigated by UV-Vis spectroscopy. The absorption spectra of the copper complexes were compared with and without CT-DNA at 400 nm (Fig. **3**) and the binding constants of **1-4** complexes are given in Table **4**. All the complexes (1-4) show a bathochromic and hyperchromic shift with an increase in absorbance on the addition of increasing amounts of CT-DNA with respect to control (without CT DNA). These absorbance values were used to calculate the intrinsic binding constant K_b (Table **4**). There is no change in absorption studies of complexes in *Tris* buffer solution, suggesting that these are stable [25]. The calculated K_b values of **1-4** complexes are in the range of 4.2 - 7.6 x 10^4 M^{-1} (Table **4**) with similar binding affinity to reported copper tetrazole derivative complexes [26]. The **1-4** complexes with CT DNA interaction are through planar phenanthroline and pyridyl tetrazole ring likely *via* π-stacking interactions. As per the literature shift and K_b values of **1-4** complexes is similar to *cis*-platin, suggesting binding of **1-4** complexes to CT-DNA by electrostatic or groove binding [27, 28].

Table 4. Binding constant and absorption (λ_{max}) data of 1- 4 complexes with respect to CT DNA.

Complex	λ_{max} /nm		Δλ/nm	K_b / M^{-1}
	Free	Bound		
1	441.6	443.4	1.8	7.6 x 10^{-4}
2	440.8	441.8	1.0	6.3 x 10^{-4}
3	439.2	439.6	0.8	5.6 x 10^{-4}
4	438.4	438.8	0.4	4.2 x 10^{-4}

*H% = Hyperchromism; Δλ = Bathochromic shift.

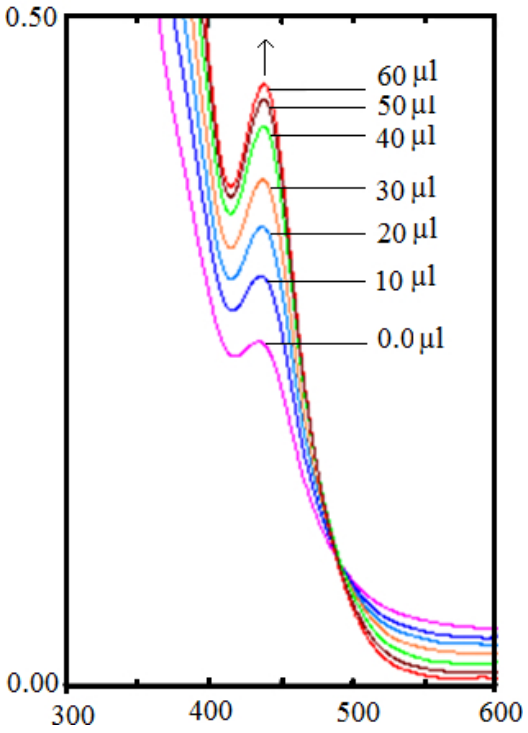


Fig. (3). Absorption spectra of complex **1** with increasing concentration of CT-DNA.

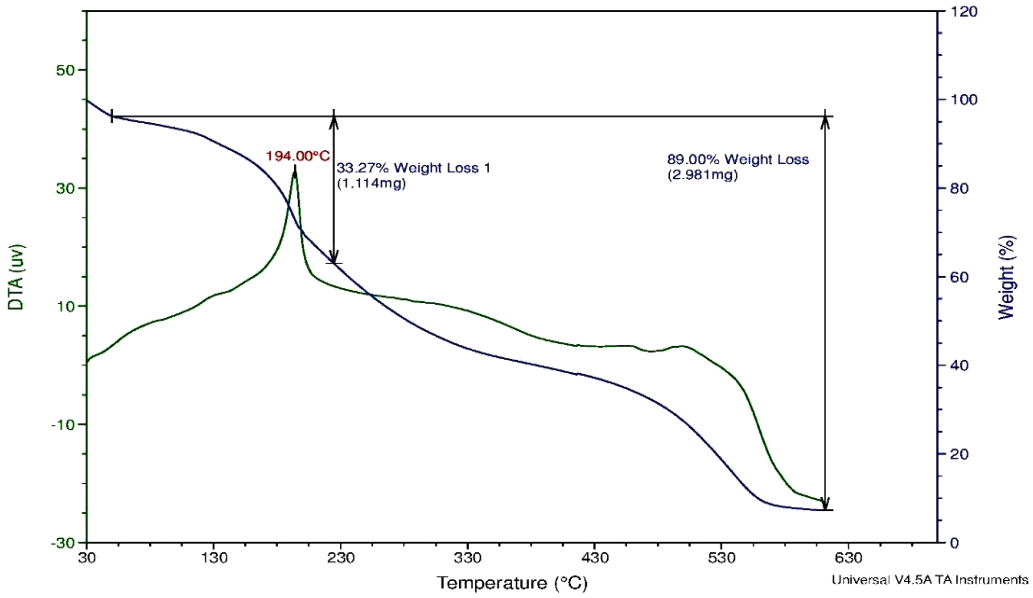


Fig. (4). TGA and DTA curve of complex **1**.

4. THERMAL DENATURATION STUDIES

The Thermal Denaturation studies of **1-4** complexes with CT DNA are carried as per our previous studies and literature [29]. The transition temperature (T_m) of CT DNA from helix form to coil from is monitored by absorbance studies at 260 nm with concentrations at [DNA]/[complex] = 25 of DNA bases. The T_m of free CT-DNA is observed at 60.1°C under employed experimental conditions. Under similar conditions, the addition of complexes **1-4** to CT DNA increased T_m values by 4, 3, 3 and 3°C respectively, indicating that **1-4** complexes stabilize the double helix of DNA.

5. CYTOTOXICITY STUDIES

XTT assay (2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2 H-tetrazolium-5-carboxanilide) was used to measure the cell proliferation activity at different concentrations of **1-4** complexes (5, 10, 50 and 100 µM) and tested in triplicate for 48 h [30, 31]. These results are expressed in average-standard devi-

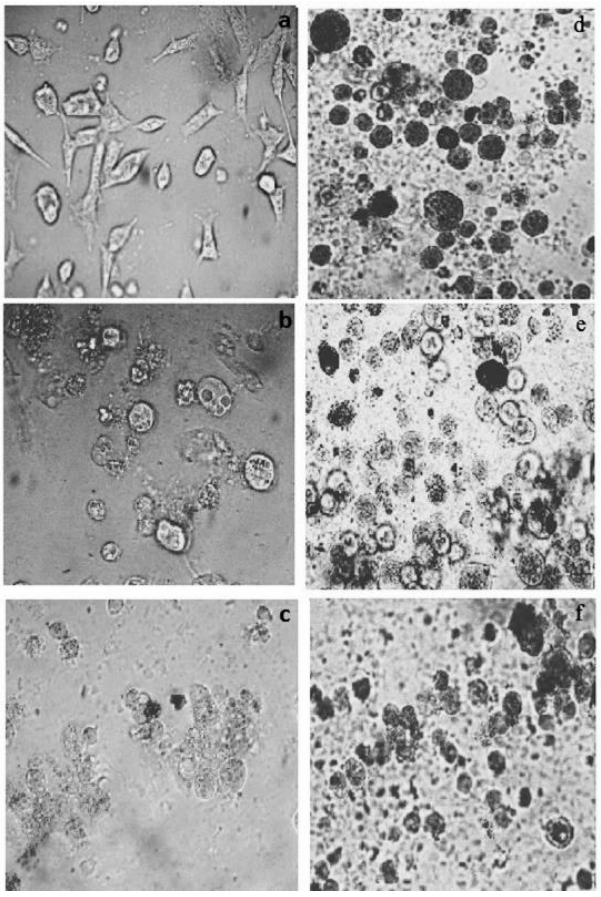


Fig. (5). Cytotoxic activities of a)–c) Cis platin(control), d)–f) complex **1** and after 12, 24, and 48 h, respectively.

ation of two experiments obtained and *Cis* platin was used as a standard control. The IC_{50} values of the **1-4** complexes show dose-dependent effective inhibition on the growth of MCF-7 cells growth. The IC_{50} values of complexes show exc-ellent activity with 24(± 0.5); 18(± 0.5); 20(±0.5); (±0.5) and 38 (±0.8) for **1, 2, 3, 4** and *cis* platin complexes, respectively. After 72 h of the treatment of **1** on MCF-7 cell, the IC_{50} values hinder the cell growth upto 24(± 0.5) µg/ml at 5 µM conce-ntration range (Fig. **5**). It is apparent from IC_{50} values that the order inhibition is **1** > **3** >**2** > **4**.

6. INHIBITORY EFFECTS OF COMPLEXES ON THE SURVIVAL OF MCF-7 AT DIFFERENT CONCENTR-ATIONS

Survival studies of MCF -7 at different concentrations were carried out by incubating cells with complexes **1-4** constantly and washed to remove the copper complex. The cell survival was determined at the complex concentration of 5, 10, 50 and 100 µM. At these concentration, complexes **1** was able to kill 78%; 65%; 48% and 46%, respectively. All copper complexes show better toxicity at low concentration 5 µg/ml with 55%, 38% 28%; and 24%; for complex **1-4,** respectively.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise

ACKNOWLEDGEMENT

Declared none.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers Website along with the published article.

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