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CT-DNA-binding and biological activity of mononuclear copper(II) complexes with imidazo-phenanthroline ligands

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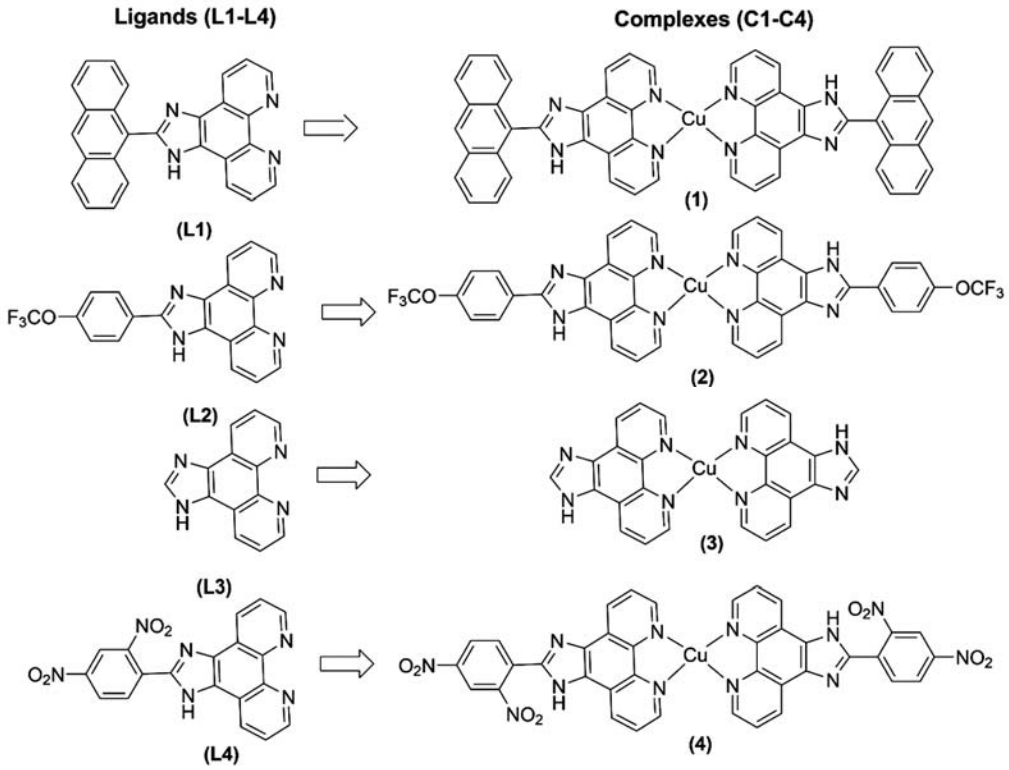
Four imidazo-phenanthroline (IP) based ligands and its corresponding copper polypyridyl complexes have been prepared $[Cu(\mathbf{L1\text{-}L4})_2]^{2+}$ and characterized by various physicochemical techniques. Herein we are reporting the CT–DNA (calf-thymus DNA) binding & anti-cancer affinity of ligands (**L1-L4**) as well as complexes (C**1–C4**). The DNA binding affinity of synthesized **C1-C4** has been carried out by using spectroscopic techniques like UV/visible, emission, molecular modeling and viscosity techniques. The obtained results are clearly indicating that all **C1-C4** complexes bind to DNA *via* intercalative mode and they possess a significant cytotoxic effect toward selected cancer cell lines (MDA-MB-231, B16-F10, DU-145 and CHO-K1).

Keywords: Cu(II) complexes, DNA-binding, Docking studies, Anti-cancer activity

Many transition metal complexes well-known to interact with DNA have been the subject of extensive research in the development of new drugs in modern medicine.[1-2] In medicinal bio-inorganic chemistry, cisplatin is one of the most effective and leading chemotherapeutic drugs against cancer, which had a metal coordination compound with non-organic units.[3-7] It is not successful against numerous normal types of cancer growth, medicine resistance is normal and an unpardonable scope of symptoms, which can contain hair loss, damage of nerve, nausea, etc. To conquer these limitations, a few organic ligand-dependent metal-based drugs have been synthesized and tried against various cancer cell lines. These complexes have a tendency to cause fewer & showing less side effects when compared to existing platinum-based drugs. De Sousa et al. have reported that *cis*-Ru(II) complex exhibits significant DNA-binding, antibacterial and pharmacological potential agents[7]. Li et al. have executed ruthenium complexes as potential drugs for eukaryotic cells and intracellular accumulation in eukaryotic and bacterial cells[8]. Liu et al. have reported that the cis-Ru(II) complexes were showing significant toxic effects on eukaryotic cells[9]. Lin et al. have reported that ruthenium complexes are new applications for clinical antitumor drugs to treat tumors synergistically[10]. Currently, copper(II) complexes seem to be the best alternatives to platinum-containing anti-cancer drugs (ex: cisplatin) and ruthenium-containing anti-cancer drugs (ex: NAMI-A). Because copper is a fundamental trace mineral present on the whole body tissues and blood cells. It additionally helps to keep healthy the immune system, blood vessels and nerves system. Copper may also useful as antioxidant, reducing free radicals which can damage DNA and cells. As per previous reports most of the Cu-based metal complexes act as a potential anticancer and pharmacological agents etc.[11-16] and both cost-wise and toxicity-wise copper is the best metal compared to platinum and ruthenium. To the continuation of our work we have designed and synthesized new copper complexes as anticancer drugs to target DNA[17], here we are discussing four copper(II) complexes as anticancer agents and binds to DNA via intercalative mode (Scheme 1).

Materials and Methods

All solvents and chemicals/reagents were acquired monetarily and utilized moving along without any more decontamination except if generally noted.



Scheme 1 — Structures of Imidazo-phenanthroline (IP) based ligands (**L1-L4**) and synthesized Cu(II) complexes (**C1-C4**)

$Cu(ClO_4)_2 \cdot 6H_2O$ and CT-DNA and 1, 10-phenanthroline monohydrate ($C_{12}H_8N_2$. H_2O) were brought from Sigma-Aldrich/Merck.

Analytical measurements

Perkin Elmer UV-visible spectrophotometer lambda-750 model was used for recording absorption spectra, Bruker 400 MHz model was used for ^1H NMR spectra with DMSO (d^6) as the solvent & internal standard $Si(CH_3)_4$ (tetramethylsilane) at room temperature. The electronic absorption spectra of complexes (**C1-C4**) were recorded both with and without DNA variable concentration in buffer solution and for complete soluble need to add a drop of DMSO. The buffer solution composition is 10 mM Tris HCl and 50 mM NaCl in buffer (pH 7.1). The absorption titration test finished by keeping up at a consistent concentration of complexes (**C1-C2**) (2.0×10^{-4} M) and with expanding the concentration of DNA over the range (0–6.0×10^{-4} M). The blended complex–DNA arrangement was permitted to equilibrate for 10 min at room temperature.

Preparation of stock solution and in vitro cell culture experiments

The required quantity of respective ligands **(L1-L4)** and complexes **(C1-C4)** were weighed and dissolved in DMSO (molecular biology grade) to prepare a stock solution of 10 mM concentration. Each time we used fresh stock solutions for all studies.

The cytotoxic studies of in vitro for synthesized **C1-C2** were assessed using standard MTT (3-(4, 5-Dimethylthiazole-2-yl)-2, 5-Diphenyltetraazolium bromide) analysis. Selected cells ((MDA-MB-231, B16-F10, DU-145 and CHO-K1) were placed in 96-well microassay culture plates ($8x10^3$ per well) in 200 µL in a 5% CO_2 incubator were grown 12 h. (overnight) at ~37 °C. Control wells were organized by addition of culture medium (200 µL). Doxorubicin and mitomycin & DMSO used as a +ve as well as vehicle controls. The absorbance was examined at 620 nm by using a 96-well plate reader. The standard solutions of the synthesized **C1-C2** were prepared in DMSO solvent, the % of DMSO solvent was kept in the range of 0.1–2 % because until 2% of DMSO found to be nontoxic to the cells. Results were collected for 3-repeats each to get the mean qualities. The ic50 values were dictated by plotting the % feasibility vs concentration on a logarithmic diagram and perusing the concentration at which 50% of cells remained reasonable with respect to control.[18]

Chemical synthesis

Synthesis and characterization: 1,10-phenanthroline-5, 6-dione was synthesised as per literature[18] **IP** ligands like: **AIP**, **Tfm-PIP**, **IP** and **Dn-PIP**, were synthesized by mixing of 1,10-phen-5,6-dione with respective selected aldehydes as per the previous literature.[20-22] The copper(II)polypyridyl complexes were synthesized readily by reacting copper perchlorate with the appropriate ligands; the complexes **(C1 to C4)** formed as the corresponding salts. A detailed procedure for the synthesis and characterization of ligands, as well as complexes, has given below.

*Preparation of 2-(anthracen-9-yl)-1H-imidazo[4,5-f][1,10]phenanthroline (**AIP**):* The reaction taken place with the mixture contains 0.53g (or) 2.50 mmol of 1,10, phenanthroline 5,6-dione[19] with anthracene-9-carbaldehyde (0.62 g, 3.02 mmol), add 15 mL of glacial acetic acid and then add 3.88 g (or) 50.00 mmol ammonium acetate the mixture was refluxed for three hours[18-22] and allow to cool upto room temperature than add water for dilution. Then add liquor ammonia slowly until to get yellow PPT then filtered, washed with water for several times then dray it for analysis. The product was gained about 79% yield; ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm): 9.12-8.80 (m, 4H), 8.12-8.16 (m, 2H), 8.08 (s, 1H), 7.88-7.77 (m, 4H), 7.61-7.49 (m, 4H). ESI-MS: m/z = 397 (M+H) peak observed (Supplementary Data).

*Preparation of 2-(4-(trifluoromethoxy)phenyl)-1H-imidazo[4,5-f][1,10]phenanthroline (**Tfm-PIP**):* The reaction taken place with the mixture contains 0.53g (or) 2.50mmol of 1,10, phenanthroline 5,6, dione[19] with 4-(trifluoromethoxy) benzaldehyde (0.57 g, 3.02 mmol), add 15 mL of glacial acetic acid and then add 3.88 g (or) 50.00 mmol ammonium acetate the mixture were refluxed for three hours[18-22] and allow to cool upto room temperature than add water for dilution. Then add liquor ammonia slowly until to get yellow PPT then filtered, washed with water for several times then dray it for analysis. The product was gained about 76% yield; ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm): 9.05 (d, J = 4.1 Hz, 2H), 8.92 (d, J = 8.0 Hz, 2H), 8.38 (d, J = 8.6 Hz, 2H), 7.81-7.78 (m, 2H), 7.58 (d, J = 8.4 Hz, 2H); ESI-MS: m/z = 381 (M+H) peak observed (Supplementary Data).

*Preparation of 1H-imidazo[4,5-f]-[1,10] phenanthroline (**IP**):* The reaction taken place with the mixture contains 0.53 g (or) 2.50 mmol of 1,10, phenanthroline 5,6, dione[19] with formaldehyde (0.09 g, 3.02 mmol), add 15 mL of glacial acetic acid and then add 3.88 g (or) 50.00 mmol ammonium acetate the mixture were refluxed for three hours[18-22] and allow to cool upto room temperature than add water for dilution. Then add liquor ammonia slowly until to get yellow PPT then filtered, washed with water for several times then dray it for analysis. The product was gained about 72% yield; ^{1}H-NMR (DMSO-d6, 300 MHz, δ ppm): 8.19-8.17 (m, 2H), 7.98-7.96 (m, 2H), 7.61 (s, 1H), 6.99-6.95 (m, 2H). ESI-MS: m/z = 221 (M+H) peak observed (Supplementary Data).

*Preparation of 2-(2,4-dinitrophenyl)-1H-imidazo[4,5-f][1,10]phenanthroline (**Dn-PIP**):* The reaction taken place with the mixture contains 0.53 g (or) 2.50 mmol of 1,10, phenanthroline 5,6, dione[19] with 2,4-dinitrobenzaldehyde (0.59 g, 3.02 mmol), add 15 mL of glacial acetic acid and then add 3.88 g (or) 50.00 mmol ammonium acetate the mixture were refluxed for three hours[18-22] and allow to cool upto room temperature than add water for dilution. Then add liquor ammonia slowly until to get yellow precipitate then filtered, washed with water for several times then dray it for analysis. The product was gained about 72% yield; ^{1}H-NMR (DMSO-d6, 300 MHz, δ ppm): 8.19-8.17 (m, 2H), 7.98-7.96 (m, 2H), 7.61 (s, ^{1}H), 6.99-6.95 (m, 2H). ESI-MS: m/z = 387 (M+H) peak observed (Supplementary Data).

Synthesis of Copper (II) complexes

*Complexes (**C1-C4**):* It was synthesized by the following reported procedure.[23] The mixture of commercially available $Cu(ClO_4)_2$. $6H_2O$ (0.1 mmol) of with 0.2 mmol of AIP ligand in 15 ml of solvent (methanol) under stirring about 2 h at room temperature. The precipitate which was formed was separated from mixture, washed with methanol (cold condition) then dried under vacuum; FTIR (KBr): 3445, 1607, 1543, 1407, 1358, 1083, 731, 621 cm^{-1}; ESI-MS: m/z = 856 $(M)^+$ peak observed (Supplementary Data). Calculated CHN analysis for complex **1** are: C 61.25, H 3.08, N 10.58 and obtained are C 60.1, H 2.8, N 9. 8. The synthesis of [Cu(Tfm-PIP)$_2$] complex is similar to above procedure and analytical data for characterisation of complex is FT-IR (KBr): 3079, 1611, 1522, 1482, 1458, 1260, 1211, 1098, 810, 727, 621 cm^{-1}; ESI-MS: m/z = 823 $(M)^+$ peak observed. Calculated CHN analysis for complex **2** are: C 46.79, H 2.20, N 10.9 and obtained are C 44.9, H 1.9, N 9.2. The synthesis of *[Cu(IP)$_2$] complex* is similar to above procedure and analytical data for characterisation of complex is FT-IR (KBr): 3123,

1610, 1538, 1417, 1358, 1112, 1083, 813, 730, 625 cm^{-1}; ESI-MS: m/z = 503 $(M)^+$ peak observed (SI). Calculated CHN analysis for complex **3** are: C 44.2, H 2.34, N 15.9 and obtained C 42.9, H 2.1, N 12. 2. The synthesis of $[Cu(Dn\text{-}PIP)_2]$ complex is similar to above procedure and analytical data for characterisation of complex is FT-IR (KBr): 3378, 3073, 2924, 1603, 1527, 1349, 1114, 1079, 810, 735, 625 cm^{-1}; ESI-MS: m/z = 835 $(M)^+$ peak observed (Supplementary Data). Calculated CHN analysis for complex **4** are: C 43.9, H 1.98, N 16.18 and obtained C 42.7, H 2.0, N 15. 2.

Results and Discussion

DNA-Binding characteristics of complexes

Analysis with absorption spectroscopy technique

The drug molecule interaction with CT-DNA is mainly *via* two modes that were intercalative mode and non-intercalative mode, in detail (i) major/minor groove binding (ii) intercalative binding in the middle of the base sets of DNA and (iii) electrostatic binding with the negatively-charged nucleic sugar.[24-25] The absorption spectra of the synthesized **C1-C4** were analyzed within the absorption range between 200 to 800 nm in tris buffer at required concentrations of complexes (**C1-C4)** as shown in Fig. 1. The absorbance of a solute depends linearly on its concentration and therefore, absorption spectroscopy is preferably very well-suited for quantitative measurements. Absorption titrations were accomplished by maintaining a consistent concentration of **C1-C4** while increasing the concentration of the CT-DNA (0–200 μM) until the saturation point was recognized. The reaction solution needs to blend carefully and be permitted to stand about 3 to 5 minutes before each run of the scan. The characteristic peaks of π–π* ligand transitions obtained in expected UV-region and MLCT (metal-ligand charge transfer) peaks at the visible region. At the saturation point, there is no change in intensity by the addition of DNA. In the quantitative analysis, we were plotting that the $\varepsilon_0/\varepsilon$ *vs* [DNA], ε_0 & ε are the hypochromism intensities of **C1-C4** without and with DNA. The given binding constants (K_b) were determined by using the following well-known equation, as provided in the literature.[26]

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/(Kb(\varepsilon_b - \varepsilon_f)) \quad \ldots(1)$$

At a given particular DNA concentration ε_a is the extinction coefficient obtained for MLCT, the extinction coefficient of DNA is ε_f and the extinction coefficient of Cu(II) complex is ε_b for completely bound to CT-DNA. In graph plots of [DNA] versus $[DNA]/(\varepsilon_b-\varepsilon_f)$, the K_b is shown by the ratio of the slope to intercept. The non-linear least-square investigation was performed by using Origin lab software. The obtained K_b values for selected copper complexes are 3.68×10^4, 2.58×10^4, 3.05×10^3 and 1.38×10^4 M^{-1} for **C1, C2, C3** and **C4**, respectively.

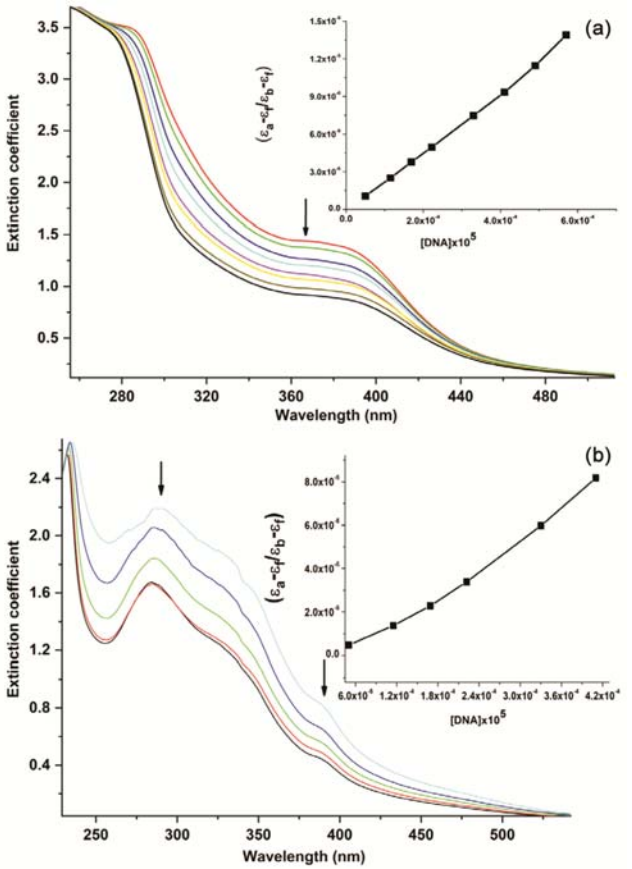


Fig. 1 — UV-visible absorption titration spectra of complex **C1** (a) and **C2** (b), [that of **C3** and **C4** are given in Supplementary Data] in the absence (top) and presence (lower) of DNA. The arrow shows that by adding the DNA solution (increasing DNA conc. in the reaction mixture) the absorbance changes

Fluorescence Spectra

In this spectroscopy, we can measure both excitation and emission spectra of the selected sample. The emission intensity is directly proportional to the concentration of the sample. Usually, metal complexes bind to DNA they show changes in fluorescence spectra. In this experiment, we took a fixed concentration synthesized complex in solution (10 μM) and to this adding DNA with varying concentration (0, 5, 10, 100 μM) and recorded the

fluorescence spectrum. The luminescence of selected **C1-C2** at ambient condition shows λ_{max} at ~250−750 nm, and when it interacts with CT-DNA was observed with luminescence. The emission titration results for complex **4 (A)** and complex **2 (B)** when binding with CT-DNA are presented in Fig. 2. Continue supply of DNA solution, the emission intensities increase for these complexes, showing their strong DNA binding capacity (for complexes **C1** and **C3** (Supplementary Data)).

Fluorescence quenching

Quenching experiments were studied using benchmark quencher $[Fe(CN)_6]^{4-}$ for further support. Without complexes were efficiently quenched with a quencher and the combination of DNA + complex, showing very less quenching effect. This may possibly due to strong negative (–ve) charged ferrocyanide ion would have repelled by the negative charge of the DNA phosphate backbone and hindered the quenching ability of the bound complexes. This can be further illustrated by the fact that interacted cations of the copper(II) complexes (Cu(II)) are protected from the negative charged bound quencher by the same charged DNA phosphate groups and hindered quenching of bound complex.[27-28] The binding affinity can be determined by the slope of the curve. The quenching constant K_{sv} determined with the help of previous reports[29] (Eqn (2)).

$$I_0/I = 1+K_{sv}[Q] \quad \ldots(2)$$

where I_0 and I are the fluorescence intensities in the absence and presence of ferrocyanide (quencher), concentration of the quencher is Q and linear Stern-Volmer is $K_{sv.}$ In this plot of I_0/I vs [Q], K_{sv} is the slope. The ferrocyanide quenching plot for complex **1** in the absence, presence and excess of DNA is shown in Fig. 3.

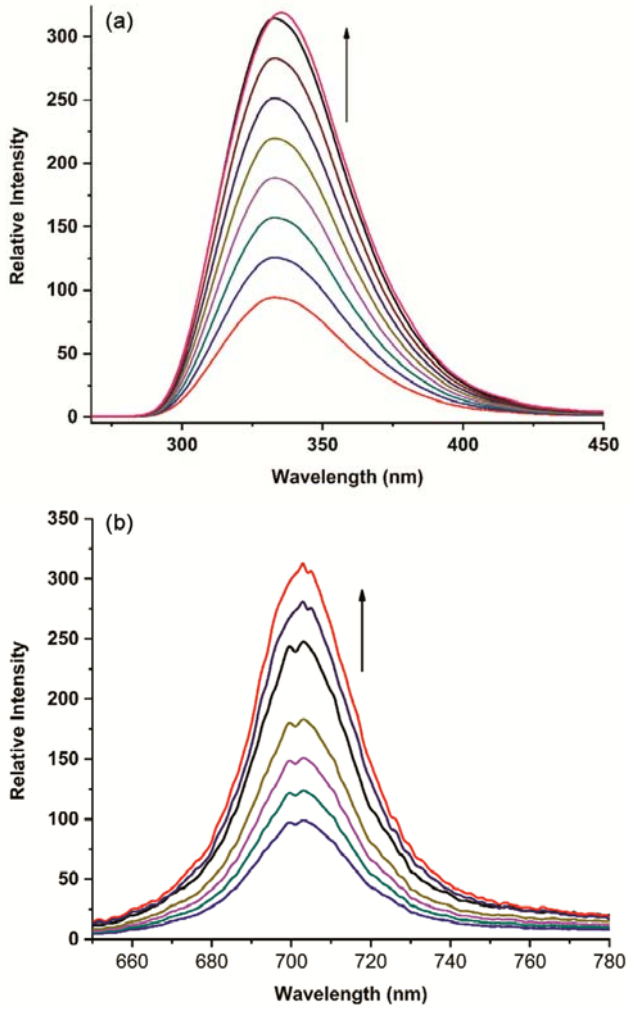


Fig. 2 — Fluorescence emission intensity spectra of complex **4** (a) and complex **2** (b) in tris buffer with addition of DNA solution, where [**C1-C4**] = 10 μM, [DNA]/ **C1-C4** = 0, 5, and 10. The arrows show that with increasing concentration of DNA the emission intensity is also increasing

Viscosity measurements

Viscosity of a liquid is the resistance of the liquid to flow. Simply a liquid having less viscosity may flows faster due to the minor friction between the molecular faces when it is in motion and more viscosity means the molecular faces have more internal friction. In this particular analysis, the viscosity technique was utilized for further confirmation of mode of interaction between

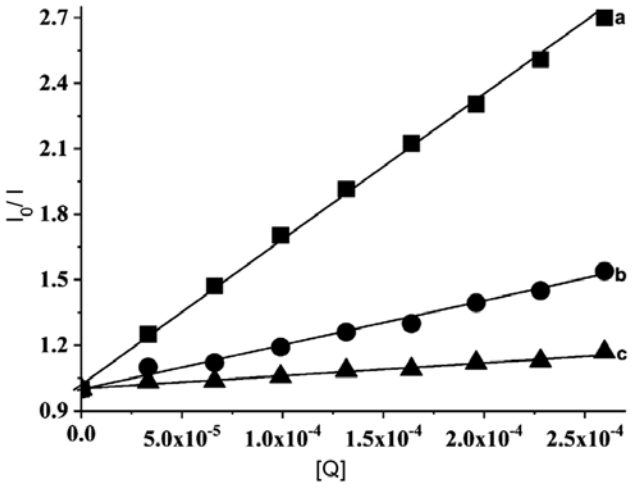


Fig. 3 — Emission quenching of complex **C1** with $K_4[Fe(CN)_6]$ in the absence (a) and presence (b) [Cu] =20 mM and excess of DNA (c)

synthesized copper(II) complexes (**C1-C4**) and DNA. As per Satyanarayana et al., if DNA length increases when drug accomodated in between the base pairs at intercalation sites, it will increase the viscosity of complex + DNA solution and this is a strong property of intercalation binding mode of complexes. On the other hand, complexes that can bind through groove generally cause less or no variation in viscosity values.[30a-b] From results obtained on relative viscosity values of DNA + complexes enhances with increasing the concentration of the complexes revealed that the intercalation mode of binding with DNA (Fig. 4). It is due to more planarity of ligands with corresponding complexes (**C1, C2 & C4**) and are showing more intercalative values than **C3** which is having less planar aromatic ligand (PIP). This is in good agreement with the obtained results from UV-visible/fluorescence emission intensity spectra.[30a-b]

Molecular docking studies

Molecular Modelling Technique (MMT) can be a very useful process in new drug discovery. It mainly includes theoretical analysis and computational methods. The docking is an analysis in MMT, which gives the coordination of one to other molecules when binding each other and form a stable complex.[31] Based on the in vitro results, we have performed the molecular docking studies, in which we selected a topoisomerase II-A bound with G-segment of DNA (PDB ID: 2RGR) from protein data bank (PDB), as the prepared complexes show evidence in vitro results that are more tend to bind the DNA. DNA-topoisomerases enzymes are a major target for cancer drugs and show a very important role in the relaxation of DNA. In the DNA replication process, relaxation of DNA is the most important step. DNA-topoisomerase reduces binding to transient enzyme-DNA complexation and results in inhibition of DNA replication.

Protein receptor is optimized by removing all the heteroatoms, water molecules using discovery studio 4.5 clients. Polar hydrogen atoms, Kolman charges were added in auto dock. The complex structures were drawn and cleaned in chemsketch added gasteiger charges to it in auto dock and optimized for docking in autodock vina. A grid was created in protein for synthesized ligands binding with optimized X, Y and Z coordinates, this program was kept running in order brief for a best fitting model in auto dock vina. The complexes C**1-C4** showing binding affinity in between 22 to 25 kcal/mol and the results are supporting that synthesized complexes are strongly binding to DNA through intercalative mode, which can correlate with viscosity results. (Fig. 5). The scores fitness values in the range 22 to 25 kcal/mol represent intercalation mechanism in between DNA and complexes due to some van der Waals forces and hydrogen bonds. Correlation between spectroscopic investigation and docking results clearly indicate about the intercalative

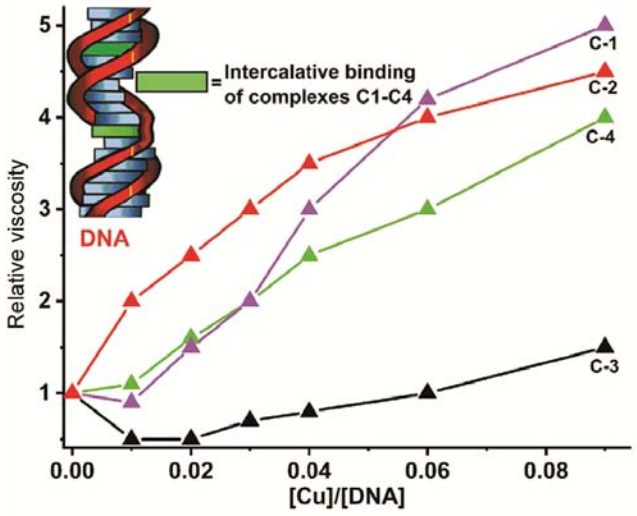


Fig. 4 — Upon addition of complexes (**C1-C4)** the effect of viscosity of DNA increases at room temperature and models for intercalative binding of complexes (right) with DNA

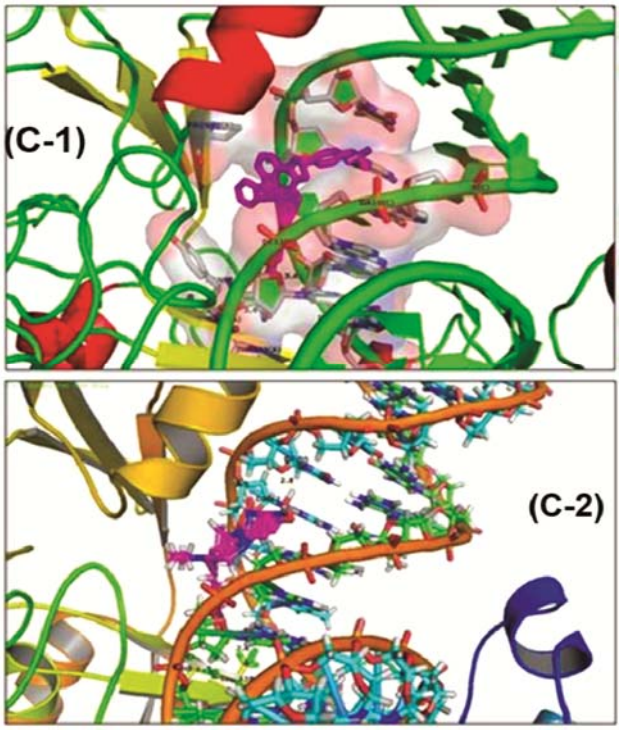


Fig. 5 — Docking models of complex **C1** and complex **C2**

Table 1 — The IC50 value of lignads (L1-L2) as well as copper complexes (C1-C4) in comparison with recently reported copper complexes (treatment duration: 24 h)

S. No.	Test compound	IC50 values (μM)				
		MDA-MB-231	SKOV3	MCF7	DU145	HepG2
1	Complex-1	7.2± 0.72	8.6± 0.66	8.2± 0.41	5.2± 0.37	8.8± 0.37
2	Complex-2	7.9± 0.57	6.9± 0.46	7.6± 0.17	4.9± 0.24	7.7± 0.47
3	Complex-3	105.6± 0.43	9.8± 0.52	20.6± 0.26	7.7± 0.32	13.2± 0.38
4	Complex-4	8.1± 0.56	10.7± 0.45	7.9± 0.74	6.3± 0.58	8.6± 0.47
5	Ligand-1	19.9± 0.23	15.1± 0.12	14.8± 0.34	10.7± 0.72	19.5± 0.37
6	Ligand-2	12.7± 0.32	6.6± 0.21	8.7± 0.45	5.5± 0.26	13.4± 0.67
7	Ligand-3	74.3± 0.44	67.6± 0.32	16.4± 0.21	32.5± 058	10.9± 0.76
8	Ligand-4	20.8± 0.66	NA	17.4± 0.12	23.7± 0.77	88.3± 0.56
Standard Drug- Doxorubicin		0.7 ± 0.12	0.8 ± 0.14	0.7 ± 0.15	0.6 ± 0.11	0.8 ± 0.12

Table 2 — IC50 value of lignads (L1-L2) as well as copper complexes (C1-C4) in comparison with recently reported copper complexes (treatment duration: 48 h)

S. No.	Test compound	IC50 values (μM)				
		MDA-MB-231	SKOV3	B16-F10	DU145	CHO
1	Complex-1	2.4 ± 0.66	7.3 ± 0.81	7.5 ± 0.0.74	4.5 ± 0.64	10.7 ± 0.60
2	Complex-2	1.6 ± 0.51	6.3 ± 0.60	8.2 ± 0.65	3.2 ± 0.53	4.6 ± 0.55
3	Complex-3	2.7 ± 0.37	7.7 ± 0.57	7.8 ± 0.84	4.4 ± 0.67	4.8 ± 0.63
4	Complex-4	2.4 ± 0.43	7.6 ± 0.62	8.5 ± 0.72	4.9 ± 0.70	1.5 ±0.37
5	Ligand-1	4.9 ± 0.58	10.3 ± 0.67	10.4 ±0.87	6.8 ± 0.77	6.4 ± 0.51
6	Ligand-2	2.1 ± 0.40	6.1 ± 0.73	11.9 ± 0.54	5.1 ± 0.66	2.0 ± 0.49
7	Ligand-3	8.1 ± 0.64	40.3 ± 0.52	10.9 ± 0.49	9.3 ± 0.74	4.8 ± 0.74
8	Ligand-4	5.8 ± 0.71	182.5 ± 1.4	15.0 ± 0.57	6.0 ± 0.58	2.7 ± 0.58
Standard Drug- Doxorubicin		0.7 ± 0.22	0.8 ± 0.21	2.1 ± 0.24	0.7 ± 0.20	-
Mitomycin C		-	-	-	-	11.3 ± 0.33

interaction in between selected complexes (**C1-C2**) & DNA, which are important for the design of new drugs that are more useful for understanding the mechanism of drugs at the molecular level.

Cytotoxicity activity

As per the reports on the cytotoxicity of copper complexes[17], we have screened the synthesized ligands (**L1-L4**) as well as prepared complexes (C**1-4C4**) for their anticancer activity. The activity of ligands and complexes were tested against five different cell lines along with doxorubicin and mitomycin as positive control and all of them showed reasonable to decent anticancer effects. The collected results are in Table 1 for 72 h, and in Table 2 for 27 h duration. The complexes whose IC50 values are showing to be lower and closer to the reference drugs like doxorubicin & mitomycin and can be considered to have decent anticancer potential. By comparing the IC50 values of ligands and complexes, we can clearly understand that the complexes are more active than corresponding ligands in most of the cases.

Conclusions

Four novel $[Cu(\mathbf{L1\text{-}L4})_2]^{2+}$ complexes were prepared with imidazo-phenanthroline (IP) based ligands which can act as an efficient DNA-binders and characterized by various physicochemical techniques. Based absorption/emission spectrum titrations experiment results that complexes (**C1-C4)** showing hypochromic/hyperchromic effects by addition of CT-DNA indicating that strong binding with K_b values ~ 10^4 and quenching studies further supports that strong binding of the complexes to DNA. Photoactivated cleavage experiment clearly indicates the ability to cleave the circular DNA. Viscosity measurements and molecular modeling technique are clearly indicating the strong binding of these complexes with DNA through intercalitive mode. The synthesized complexes possess a significant cytotoxic effect toward several cancer cell lines **MDA-MB-231**, **B16-F10, DU-145** and **CHO-K1**. The obtained results are opening a new venture to design new drugs for cancer lines.

Supplementary Data

Supplementary Data associated with this article are available in the electronic form at http://nopr.niscair.res.in/jinfo/ijca/IJCA_60A(01)37-44_SupplData.pdf. The supplementary data contains the information relating to characterization of the

synthesized ligands and copper complexes and also the list of abbreviations used in this article. Fig. S1 and S2 are related to the absorption and emission spectra, respectively, of **C1-C4**. Fig. S3 to S6 related NMR spectrum of ligands and copper complexes (**C1-C4**). Fig. S7-12 related is related to the mass spectrum of ligands and complexes (**C1-C2**). Fig. S13 is related to photoactivated cleavage of pBR 322 DNA by copper complexes.

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