Nat. Volatiles & Essent. Oils, 2021; 8(6): 1672-1685

NVEO
Natural Volatiles &
Essential Oils

Binding Of Dna And Cytotoxicity Of Two Cu (Ii) Complexes With Co- Ligands

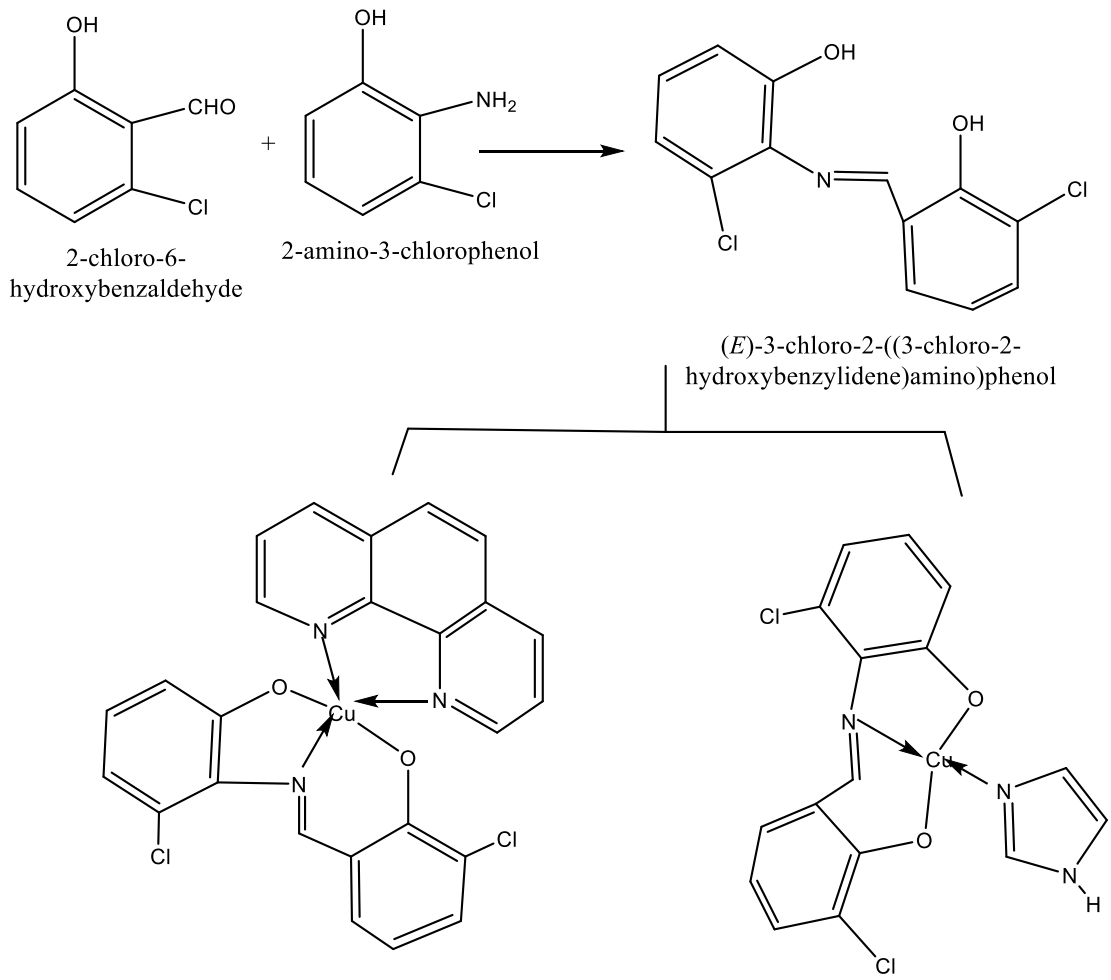
Muhammed Abdalhasan Shallal[1] , Rehab Kadhim Al-Shemary*[2]

[1]Ministry of Education, Administrative of DhiQar, Iraq

[2]Department of Chemistry, College of Education for Pure Science, Ibn Al-Haitham, University of Baghdad, Iraq

Abstract

The compounds [CuL(phen)] and [CuL(z)] are synthesized, characterized, and tested for biological activity (H2L=3-chloro-2-((3-chloro-2-hydroxybenzylidene)amino)phenol, Imz = imidazole , phen = 1,10-phenanthroline, and). Intercalative DNA binding is cytotoxic and anticancer in a biological investigation.IC50 values of [CuL(phen)] and [CuL(z)] are shown to be extremely promising for the human lung cancer A549 cell line and the breast cancer MCF7 cell line.The complexes [CuL(phen)] and [CuL(z)] appear to be more effective than numerous anticancer drugs tested in the human lung cancer A549 cell line.Most importantly, [CuL(phen)] and [CuL(z)] are substantially less hazardous to the normal cell line L132, as indicated by cell viability in the existence of these two compounds.



Scheme 1. The Schiff base and complexes are synthesised.

Keywords: UV-VIS spectra; MCF7 cell line; DNA binding; Cu(II) complex;A549 cell line

1. Introduction

In the last few decades, there has been a surge in activitiesto find or construct complexes with binding toDNA [1], cutting DNA and cytotoxicity to examine the potential of producing novel anticancer metallodrugs[2].The ligand in metallodrugs is critical for the metal complex's binding to DNA[3]. Thattargets anticancer drugs. In addition[4], the metal ion may also function in the metal complex's efficacy as a medication[5].Cu(II), for example, as a metal ion with redox activity,can stimulateprotein or DNA modification via an oxidative mechanism[6]. These complexes were found to be more potent than various anticancer medications evaluated in human lung cancer A549[7] and breast cancer MCF7 cell lines[8], according to their IC50 values[9]. In our constant search for complexes with acceptable ligands as donors with extra heterocycles, we have synthesised two Cu(II) complexes, *4*-coordinate. Biological activities include DNA binding, cytotoxicity, and anticancer action.

4. Experimental

4.1. Chemicals

Aldrich provided 2-amino-3-chlorophenol, 2-chloro-6-hydroxybenzaldehyde,and imidazole. Sigma-Aldrich (USA) provided calf thymus DNA. In contrast, Merck provided Cu(OAc)2 H2O (GR),1,10-phenanthroline monohydrate,(DMF, GR), methanol, ethanol (GR), and DMSO

4.2. Preparation of Schiff base (H2L)

2-amino-3-chlorophenol and 2-chloro-6-hydroxybenzaldehydewere made using the methoddescribed. Yield 71%. Anal Calc. for 282.12$C_{13}H_9Cl_2NO_2$: C, 54.87; H, 3.54; N, 5.12. Found: C, 55.35;H, 3.22; N, 4.96%.

4.3. preparationofComplexes

4.3.1 [CuL(ph)]

During 30 minutes of stirring at room temperature, then over 30 minutesat room temperature.H_2L in (10mL, 25°C) methanol was added to the reaction mixture with gentle stirring.A substance separated from the solution.The result was purified, rinsed completely with methanol, then with water, and dried in a vacuum.At room temperature, this chemical was recrystallised from(1:1)CH_3CN:CH_3OH to obtain good single crystals.It is worth noting that these crystals become amorphous after losing their crystallisation water after prolonged exposure (or in a vacuum).Anal. Calc. for $C_{16}H_{11}Cl_2CuN_3O_2$:C, 46.68; H,2.69; N, 10.21; Cl,17.22; Cu,15.43 .Found: C, 45.78; H,3.15; N, 10.65; Cl,17.08; Cu,14.87%. ESI-MS in CH_3CN: m/z 411.73 [M]+.

4.3.2. [CuL(z)] (2)

A solution was made by slowly adding (0.001 mol) imidazole in (15 mL) methanol to a (0.002 mol) Cu(OAc)2.H2O solution in (20 mL, 5 ˚C) methanol for 20 minutes at ambient temperature (25 ˚C) with stirring.This solution was then progressively added to a solution of H_2L(0.002 mol) in methanol (30 mL, 25˚C) at room temperature for 30 min with stirring.The solution was separated by material. The solid was filtered, washed extensively with methanol, and vacuum dried. This chemical was recrystallised at room temperature from (1:1) CH_3OH:CH_3CN, yielding good single crystals.X-ray crystallography was also used to characterise this molecule.Anal. Calc. for $C_{25}H_{15}Cl_2CuN_3O_2$: C,57.32; H, 2.89; Cl,13.53 ;N, 8.02 ,Cu,12.13. Found: C,57.11; H, 3.12; Cl,13.09 ;N, 8.43 ,Cu,12.07%. ESI-MS in CH_3CN: m/z 523.86 [M]+.

4.4. Physical measurementsFlexer SQ 300 MS Mass Spectrometer from Perkin Elmer collected mass spectra in ESI mode.An elemental analyser Perkin-Elmer 24 ˚Cwas used to perform the microanalyses (C, H, and N).In addition, a quartz dewer was used to record frozen glass spectra in liquid nitrogen.Electrochemical measurements were performed at 298K with a bioanalytical system CV-27 electrochemical analyser and a BAS type X-Y recorder under dinitrogen. In addition, A Shimadzu IR Affinity - 1 FT-IR spectrometer and a KBr pellet were used to collect infrared spectra.Furthermore, a pair of matching quartz cells with a route length of 1 cm was used to a Jasco V-570 UV/VIS/NIR spectrophotometer, record electronic absorption spectra. Finally,the cell was constructed using the platinum

working electrode, an Ag/AgCl reference electrode, and a platinum auxiliary electrode in the typical three-electrode setup.As a supporting electrolyte, ([N(n-Bu)4]ClO_4) was employed.

4.6. Methods for biological activity

4.6.1. DNA binding studies

To determine the binding constant for compounds [CuL(phen)] and [CuL(z)], with progressive quantities of these compounds, the electronic spectra of these compounds were recorded using a Jasco V-570 UV/VIS/IR spectrophotometer (CT-DNA).The titration tests were carried out in (10mM)Tris–HCl buffer with the complex concentration constant. The CT-DNA concentration varied between 0 and 40 M. (pH 7.6).

4.6.2. Cytotoxicity studies

4.6.2.1. Cell culture and treatment

NCCS Pune generously donated human lung cancer (A549) cells, human breast cancer (MCF7) cells, normal human keratinocyte (HaCaT) cells, and human lung embryonic (L132) cells. These cell lines were cultured in DMEM high glucose media with gentamycin 50 g/mL in T-25 mm NUNC cell culture flasks,10% Fetal Bovine Serum Penicillin (100 units/ml), streptomycin (100 g/ml), and.In a CO2 incubator at 37°C, (2.5 g/ml) Amphotericin B was also introduced (5% CO_2 and 95% O_2).They reached 70-80% confluence as a result, and these cells were trypsinized and sub-cultured for many tests.

Results and discussion

We investigated Schiff base reaction with $Cu(OAc)_2.H_2O$ in methanol in the presence of either imidazole or 1,10-phenanthroline as a co-ligand to form complexes[10]. The ν(C=N) band appears at 1625 cm^{-1} in the IR spectra of the free Schiff base ligand.This changes to 1603 cm^{-1} and 1607 cm^{-1}in the IR spectra of both complexes. They suggest N-azomethine chelation, deprotonation, and O-phenolate coordination to the metal ion.These are only moderately soluble in acetonitrileand methanol. However, they are quite soluble in DMF and DMSO[11].

In the visible region, the UV spectra of these two molecules are remarkably similar. However, the bands' intensities are discovered to stand out.

At roughly 676 nm (= 14,792 cm^{-1}), compound [CuL(phen)]displays a weak broad d-d band.In contrast, compound [CuL(z)] exhibits a weak ligand field band at approximately 634 nm (λ= 15,772 cm^{-1})[12]. Compound [CuL(phen)] has a large LMCT in the visible area, except for the slight ligand field transition. The charge transfer band at 467 nm (λ= 21,413 cm^{-1}) for [CuL(phen)] with 1,10-phenanthroline as the ligand is significantly stronger than [CuL(z)]with imidazole as the co-ligand at 470 nm (λ21,276cm^{-1}).Moreover, the overall intensity of [CuL(z)] is much larger than that of [CuL(z)],showing that their electrical architectures are different[13].

DNA binding studies

DNA linking studies were completed utilising spectroscopy of UV Vis.UV absorbance titration tests were performed using incremental quantities of (CT-DNA) in (10 mM) of Tris–HCl buffer (pH 7.5)[14]. At the same time, they are maintaining a constant concentration of 2x 10^{-4} M in the single complex. Figure 4 shows the absorption spectrum of complexes in the presence and absence of CT-DNA.On CT-DNA addition, both complexes appear to lower molar absorptivity of the absorption band of * (hypochromism of 47.5 % for [CuL(phen)] and 82.5 % for[CuL(z)], indicating robust DNA-complex binding.These modifications occur in complexes that intercalate with DNA via an intensive stacking contact between DNA base pairs and aromatic chromophores.To calculate the quantity rapprochement of DNA linking capacity.Using the next Eq.(1), we calculatedthe Kb of the complexes for connecting with CT-DNA (1).

$$\frac{[DNA]}{(\varepsilon_a - \varepsilon_f)} = \frac{[DNA]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)}$$

Where [DNA] denotes the CT-DNA concentration employed, and the apparent extinction coefficients of the complex are denoted by a, f, and b, i.e., Abs/[complex] in DNA existence, non-attendance, and DNA Completely bound, respectively.The plot of [DNA] versus [DNA]/(εa − εf) results in slope = 1/(εb − εf), and crossover = 1/Kb(εb − εf), respectively.Kb is determined from the proportionbetweenthe intercept and the slope. The data of Kb are 1.89 10^5 M^{-1} for compound C1 (R2 = 0.99654 for 14 points) and 3.67 10^5 M^{-1} for compound [CuL(z)] (R2= 0.99786 for 10 points), respectively. These data are similar to that ofa traditional intercalator likeEBin (20 mM) of a buffer of Tris- HCl (pH 7.33) (Kb = 1.75 × 10^5 M^{-1}). The more proportion of hypochromism(82.5%) and a more Kb data of compound [CuL(z)]compared to compound[CuL(ph)]elucidates its more tendency to ligation that arises attributed to the planar structure with 4chalets of [CuL(z)][15]. They allowed the intercalated ligand to be inserted into the DNA base pair effectively; enhanced stacking interaction increases binding ability. Table-1 shows a comparison of Kbvalues of compounds

Table 1. Values of DNA linking constant for compounds.

Compounds	K_b (M^{-1})
H2L	**1.75 × 10^5**
[CuL(ph)]	**1.89 10^5**
[CuL(z)]	**3.67 10^5**

Viscosity measurements

Both compounds' spectroscopic examinations of DNA intercalation provide additional proof.The concentration of added complex was changed to assess the viscosity of CT-DNA.When CT-DNA solution interacts with substrates that bind in the intercalative mode, the relative viscosity of the solution usually increases. Because the metal complex is inserted between DNA base pairs, the base pairs split, increasing the overall length of DNA and thus

increasing the DNA viscosity.The effect of increasing the amount of [CuL(ph)] complex and [CuL(z)]on the relative viscosity, as shown in Fig. 1, indicates a constant rise in the viscosity of the DNA, indicating that the complexes attach to DNA in an intercalative way. It's also worth noting that the viscosity rise is more pronounced in[CuL(z)]. The results support the spectral studies,which suggest that complex [CuL(z)]binds more strongly to CT-DNA.[Complex]/[DNA]

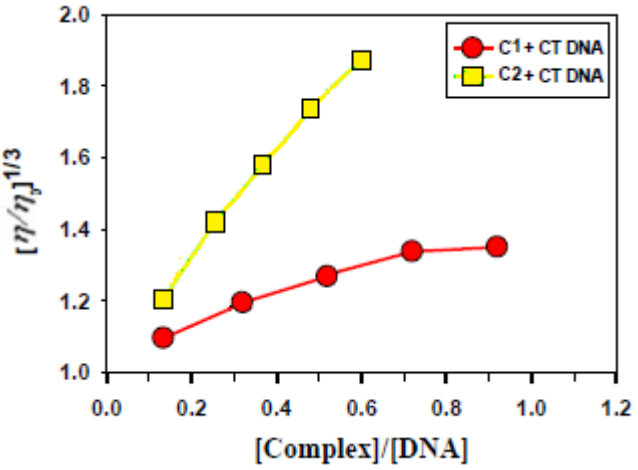


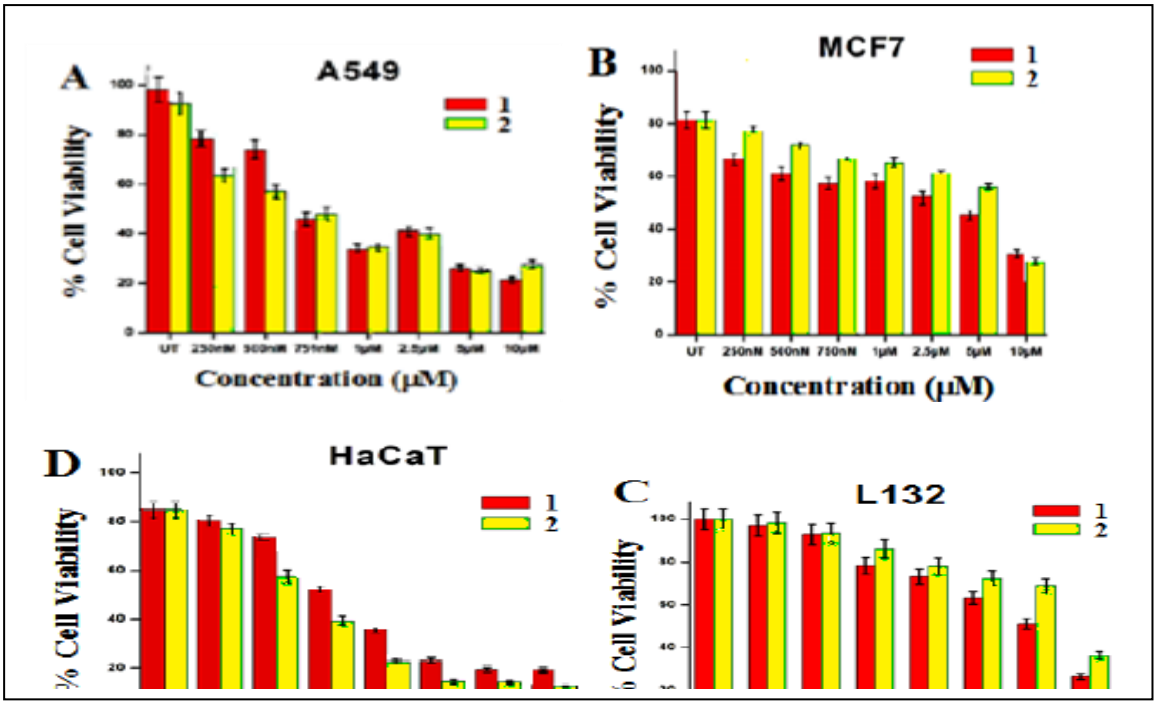
Fig.1. At room temperature in 10 mM Tris.HCl buffer, increasing the number of complexesaffect the relative viscosity of CT-DNA (100 M)

2.5.1. Cytotoxicity

The MTT assay was used to evaluate the cytotoxicity of compounds [CuL(ph)] and [CuL(z)] on lung cancer A549, breast cancer MCF7, human lung L132, embryonic normal, and human keratinocyte HaCaT standard cell lines.In addition, the vitality of the cells was assessed by converting yellow MTT into purple formazan product in living cells using mitochondrial dehydrogenase enzyme activity.Both malignant (MCF7 and A549) and conventional cell lines (MCF7 and A549) showed a concentration-dependent influence on cell viability (HaCaT, L132).

The results show that even at a low concentration of 0.30 M, the compounds are toxic to A549 cells, evidenced by their respective cell viability of 84% and 70%, respectively. At the same time, toxicity is much less in normal HaCaT cells at the same dose (cell viability is 93% for [CuL(ph)] and 89% for [CuL(z)]. Almost no toxicity was observed for L132 lung cells. Cells of A549 treated with 0.6 M of [CuL(ph)] and [CuL(z)] have a viability of 65% and 50%, respectively. That compared to 90% for L132 cells treated with the same dose of compounds. In addition, compound [CuL(ph)] exhibits similar results in normal HaCaT cells, with 95% cell viability at 0.6 M dosage. Similar results were seen when A549 cells were given a 1.25 M dosage of compounds; cell viability was reduced to 40% for both compounds.

Fig. 2. MTT assay for 1 and 2. A549 cell line, MCF7 cell line, L132 cell line, and HaCaT cell line.



On the other hand, the compounds appear to be substantially less harmful to L132 normal cells, with cell viability of 78 and 86 %, respectively, after treatment with 1 M doses of compounds. Compound [CuL(z)] was found to be highly toxic to HaCaT cells at the same 1 M dose. However, cell survival appears to be moderate (50%) when normal HaCaT cells are treated with it. When the effect of 2.5, 5, and 10 M doses of compounds on lung cancer A549 cell lines versus L132 lung normal cells are evaluated, the dramatic difference in cell viability is observed once more. Compound [CuL(ph)] is more harmful than[CuL(z)] in the MCF7 cell line over the whole concentration range examined but substantially less toxic in A549 cells.Again, a substantial difference in cell viability is seen when the effect of 2.5, 5, and 10 M doses of compounds on lung cancer A549 cell lines versus L132 lung normal cells is evaluated.Compound [CuL(ph)] is more harmful than compound [CuL(z)] in the MCF7 cell line over the whole concentration range examined but substantially less toxic in A549 cells.However, both compounds are exceedingly toxic at concentrations of 2.5 M and above towards cells of HaCaT.However, at 1.0 M, compound [CuL(ph)] is discovered to be notably less hazardous than [CuL(z)], as evidenced by their cell viabilities of 51% and 20 %, respectively.Table 2 shows the IC50 values for compounds calculated from this plot for both the cancer cell lines A549 and MCF7 and the two standard cell lines.

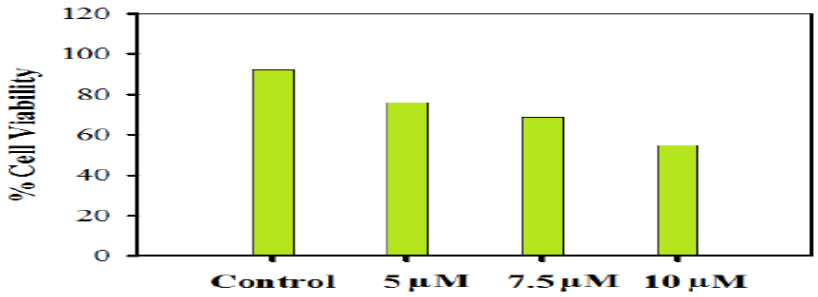


Fig. 3. Cell viability of human lung cancer cells with 5 M, 7.5 M, and 10 M free Schiff base ligand N-salicylidene-o-aminophenol The A549 cell line after 24 hours.

It should be noted that the IC50 values for A549 cells are higher than the IC50 value for cisplatin (4.5 M for A549).The IC50 values for compounds for the MCF7 cell line are somewhat higher than those reported for cisplatin (3.1M for MCF7) and significantly lower than those reported for carboplatin (35.88 M for MCF7).We recently reported a few Cu(II)/Zn(II) complexes with a carbohydrate ligand and 1,10-phenanthroline as a co-ligand. They were found to be up-and-coming anticancer agents tested for the breast cancer MCF7 cell line and the human lung cancer A549 cell line based on their IC50 values.However, this Cu (II) compounds were hazardous to the normal L132 and HaCaT cell lines.The two compounds investigated in this work, on the other hand, were shown to be substantially less hazardous to regular L132 cell lines. According to the findings, they may be helpful as anticancer therapy for the human lung cancer A549cell line.

In contrast, the two chemicals investigated in this study were determined to be significantly less dangerous to conventional L132 cell lines, emphasising their utility as anticancer medications specifically for human lung cancer A549 cell lines.

To assess the relevance of our findings, we compared the IC50 and Kb values of compounds to those of several recently published copper (II) complexes for A549 and MCF7 cell lines.According to the comparison (as indicated by lower IC values), the compounds exhibit a higher Kb intrinsic binding constant and a more cytotoxic effect in the A549 cell line. According to the comparison (as indicated by lower IC values) than mostCu(II) complexes. However, no cytotoxic effect on any standard cell line has been found. In this work, complexes were tested in both carcinogenic and noncancerous cell lines, and they showed a substantial difference in cytotoxicity in lung cancer A549 versus human lung normal cell line L132, showing their potential to operate as anticancer therapeutic agents.The therapeutic index (TI) of compounds was calculated by comparing the IC50 values of the complexes in normal and cancer cells to determine their respective antiproliferative effects on malignant vs normal cells.

Table 2. The IC50 values for compounds were obtained using the MTT assay's plot of cell viability vs concentration

Cell line	IC50 (µM) for	
	CompoundC1	CompoundC2
A549	0.73	0.64
MCF 7	7.11	9.21
L132	8.32	10.23
HaCaT	1.34	0.97

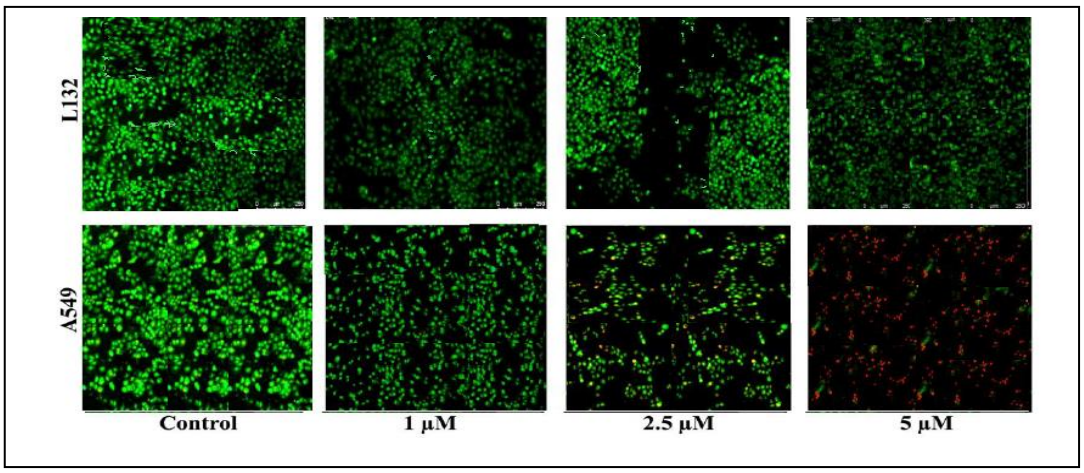


Fig. 4. AO/PI staining results for compound [CuL(ph)]using A549 and L132 cell lines. Scale bar: 250 m

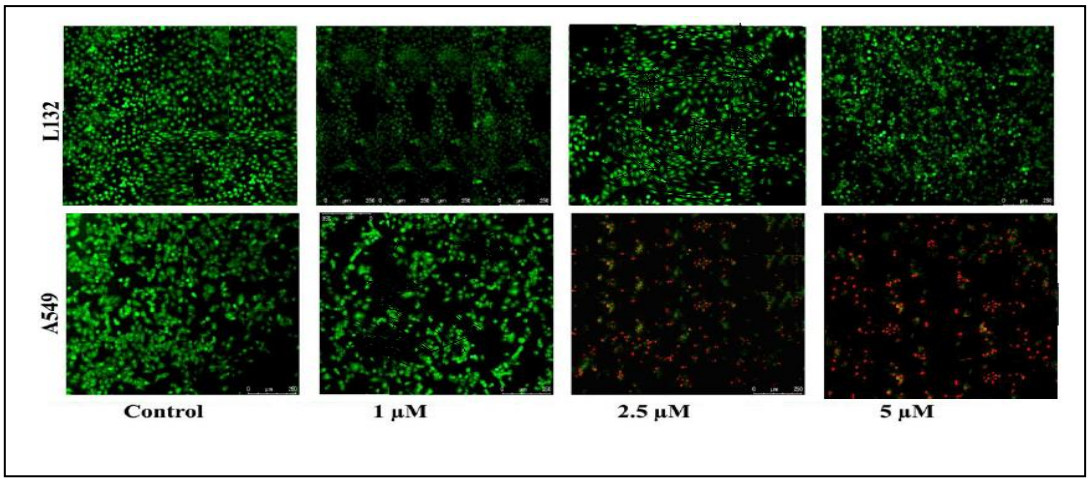


Fig. 5. AO/PI staining findings for compound [CuL(z)] using A549 and L132 cell lines. Scale bar: 250 m

2.5.2. Dual-Fluorescence Viability: A549 and L132 Cells AO/PI Dual Staining Assay

This fluorescence microscopy-based dual staining experiment is used to investigate the induction of cell apoptosis in the presence of complexes.Nucleic acid-binding dyes acridine orange (AO) and propidium iodide (PI) were used. AO is permeable to both live and dead cells, and it produces green fluorescence in all nucleated cells.On the other hand, PI only enters dead, dying, and necrotic nucleated cells with damaged membranes, staining and creating red fluorescence in all of them.When cells are stained with both AO and PI, energy transfer occurs due to Förster resonance. All dead nucleated cells shine red, while all living nucleated cells illuminate green (FRET).(a)The following morphological alterations were detected in the treated cells using this staining approach. (b) Early apoptotic cells with nuclear condensation produce orange-green fluorescence in the treatment with 2.5 and 5 M

concentrations of the respective drugs.Early apoptotic cells with nuclear condensation produce orange-green fluorescence in the treatment with 2.5 and 5 M concentrations of the respective drugs. (c) Late apoptotic cells with severely compacted or fragmented chromatin and orange to red fluorescence were detected in the treatment with 2.5 and 5 M doses of the various drugs. (d) In the case of A549 cells treated with 5 M of each of the complexes, necrotic cells fluoresce red with no evidence of chromatin fragmentation. While no such red fluorescence is seen in L132 cells with 1-5 M concentrations for compounds, implying that no or very little DNA damage occurs at these doses. The cell viability reported in their MTT assay is consistent with these findings, indicating the significant disparities in cell death in A549 and L132 cells treated with complexes. Based on these morphological alterations, both complexes may have caused cell death by apoptosis and necrosis. It should be noted that in vitro DNA binding tests revealed that the two chemicals under investigation are capable of DNA intercalation. This does not rule out the possibility that the observed cell death is caused by this intercalation, as the structural stability of these complexes once they enter the cell membrane will be crucial. Though this possibility cannot be ruled out entirely at this time, other possibilities include them being reduced to Cu(I) in the reducing environment after entering the cell membrane.

Following ligand dissociation (as evidenced by their electrochemical activity). They may either interact with the proteasome [2d] to cause apoptosis or mix with dioxygen to produce reactive oxygen species (ROS). Thus, cancer cells have (ROS) levels than normal cells, leading to oxidative breakage of DNA polynucleotide chains and apoptosis.At the same time, cancer cells do contain higher quantities of reactive oxygen species (ROS) than normal cells. However, compared to normal cells, cancer cells contain higher quantities of reactive oxygen species (ROS). With the current experimental evidence, this is the most plausible answer we can offer.

It should be noted that the cytotoxicity of the free Schiff base ligand was also studied for 24 hours in the human lung cancer A549 cell line at various dosages (2.5 M, 5 M, 7.5 M, and 10 M). With a 2.5 M concentration of the free ligand, no cell death was detected. However, with 5 M, 95.5 M, and 91 percent of the free ligand, cell viability was determined to be roughly 96.5 %t, 95.5 %, and 91 %, respectively. Thus, the free ligand can induce 9-10% cell death in the presence of a 10 M concentration of free ligand. That meaning that the free ligand can induce 9-10% cell death if the compounds undergo ligand dissociation after penetrating the cell membrane. Thus, the toxicity was seen in compounds (e.g., at dosages of 5 M and 10 M, respectively) could be attributed to the free Schiff base ligand. Otherwise, the complex as a whole should be responsible for the reported toxicity.

2.6. The outcomes of electrochemistry

A platinum working electrode was used to study the electrochemical behavior of compounds [CuL(ph)] and [CuL(z)] in DMF containing 0.1 M $[N(n\text{-}Bu)_4]ClO_4$. Both compounds' cyclic voltammetric behavior is independent of scan directions, yielding oxidation and reduction peaks at nearly identical positions regardless of scan direction. (Fig.5, panels A, B for [CuL(ph)], and panels C, D for[CuL(z)], respectively.Compound 1 exhibits a large reduction wave near 1.25 V; reversing this scan results in three consecutive oxidation waves at 0.78,

+0.839, and +1.17 V, respectively. When scanned within -1.5 - +1.5 V, reversing the scan generates reduction waves at +0.78and +0.10. On scan reversal, Compound [CuL(z)], exhibits a large reduction wave of about 1.55 V (against Ag/AgCl), a small oxidation peak at 1.25 V**,** and another oxidation peak near 0.0915 V. A positive scan shows an oxidation wave near +0.88 V,

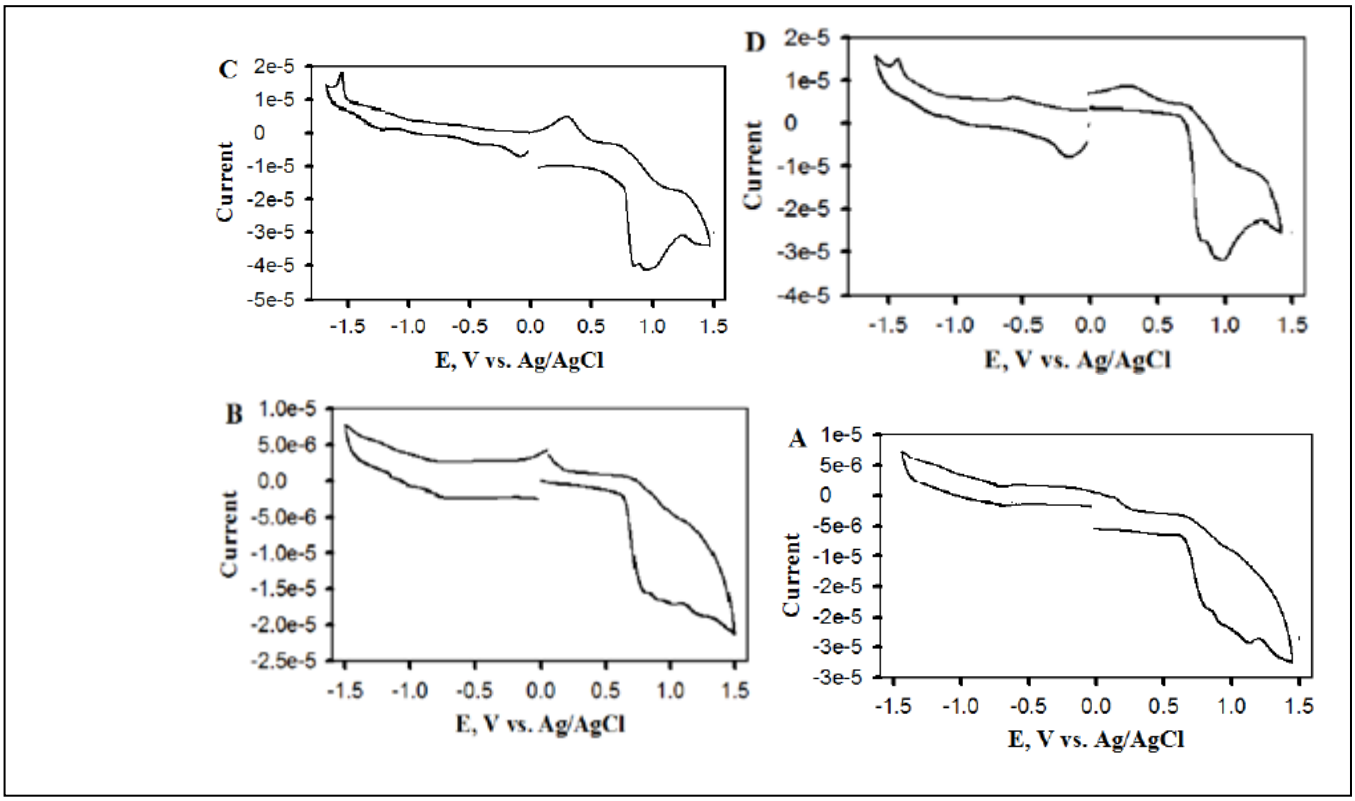


Fig. 6. The compounds (1.25 103 mol L-1) in DMF with 0.1 M [N(n-Bu)4]ClO4 at 100 mVs[CuL(ph)]. A, B for 1, C, D for [CuL(z)], respectively.followed by three

reduction waves at +1.31, +0.789, and +0.3234 V, respectively. The reduction peak at 0.0865 V is connected to the oxidation wave at +0.3235 V, showing a partially reversible redox process. Hence, the absence of any reversible or almost reversible Cu(II)/Cu(I) redox pair in these complexes' cyclic voltammograms indicates that they undergo irreversible oxidation and reduction involving both the metalcore and the ligand centre.Since the redox potentials of Cu(II)/Cu(I) complexes are dependent on the thermodynamic stability of the two oxidation states in a given ligand environment. As a result, none of them preserves the same shape in both oxidation states.

3. Conclusions

Their DNA binding studies with calf thymus DNA demonstrated that both compounds readily intercalate with binding constants of **1.89 10^5** M^{-1} and **3.67 10^5**M^{-1}. Compounds [CuL(ph)] and [CuL(z)] are cytotoxic to human breast cancer MCF7 cells and human lung cancer A549 cells in vitro. Their IC50 values suggest that they are quite potent for the A549 cell line in particular. They are also discovered to be substantially less harmful to the L132 human lung

embryonic normal cell line. The AO/PI dual-labelling data strongly suggest that these complexes exert anticancer action by activating both apoptotic and necrotic cell death pathways.

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