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SYNTHESIS, CHARACTERIZATION, ANTIOXIDANT AND ANTICANCER STUDIES OF COPPER(II) COMPLEXES OF 2-AMINOBENZIMIDAZOLE SCAFFOLD SCHIFF BASES

A. Krishnaveni[1], M. Kalanithi[1,✉], M. Karthikeyan[2], and U. Deenathayalan[3]
[1]PG and Research Centre of Chemistry, Jayaraj Annapackium College for Women (Affiliated to Mother Teresa Women's University, Kodaikanal),Periyakulam-625601,Theni, Tamil Nadu, India
[2]Department of Microbiology,PSG College of Arts & Science, Coimbatore-641014, Tamil Nadu, India
[3]Department of Biochemistry, Karpagam Academy of Higher Education, Coimbatore-641021, Tamil Nadu, India
✉Corresponding author: kalachem@annejac.ac.in

ABSTRACT
2-Aminobenzimidazole is a heterocyclic compound of significant interest in drug design and materials science due to its ability to interact with biological systems and coordinate metal ions, making it useful for both pharmaceutical and industrial applications. Novel Cu(II) complexes (L1Cu–L4Cu) were synthesised using Schiff base ligands (L1–L4) prepared by condensing 2-aminobenzimidazole with various aromatic aldehydes, including p-chlorobenzaldehyde, p-methoxybenzaldehyde, 3,4,5-trimethoxybenzaldehyde, and p-hydroxybenzaldehyde. The Schiff base ligands and their copper (II) complexes were characterised using magnetic susceptibility, molar conductance, electronic, FT-IR, Nuclear magnetic resonance, and mass spectrum analyses. Spectral analyses suggested all Copper(II) complexes have a geometry of square planar. Ligands (L1–L4) and Copper(II) complexes were examined for antioxidant behaviour using DPPH, and the findings showed that compounds containing methoxy groups exhibited higher activity than the others. Moreover, in vitro anticancer activities of the Schiff bases (L1–L4) and their Cu(II) complexes were assessed against HeLa cancer cell lines using the MTT assay. The findings exposed a hydroxyl-substituted ligand and its Cu(II) complex demonstrated marked cytotoxic activity towards the HeLa cell lines.

Keywords: Schiff Base, 2-aminobenzimidazole, DPPH, HeLa Cells, Antioxidant, and Anticancer Activity.
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INTRODUCTION

Heterocyclic ligands are the most thrust areas in medicinal and coordination chemistry.[1] In that line, the benzimidazole moiety drew the attention of researchers in the past two decades. From the research survey, it is known that the biological activity of benzimidazole scaffolds confirms the pharmaceutical nature of the benzimidazole hybrids.[2-4] The cytotoxic activity is directly related to the substitution present in the phenyl ring, the number one and number two positions. The target to inhibit also depends on the structure of the benzimidazole compound. Many of the anticancer drugs, such as Bendamustine[5], Nocodazole and Veliparib[6] cover the benzimidazole moiety. The cytotoxic activity of Benzimidazole-based motifs against cancer cells, with the structure activity analysis, has also been analysed.[7,8] Exploring the synthesis and properties of Copper(II) complexes of benzimidazole-based Schiff bases continues to be a significant research focus in bioinorganic chemistry. Metal coordination makes the ligand more biologically active due to lipophilicity. Among the metal ions, copper is a crucial metal as it is present in living organisms and is responsible for the proper functioning of organs and metabolic processes. Metal chelates of benzimidazole Schiff bases are significant for their antimicrobial,[9-11] antiulcer[12], analgesic[13] and antioxidant[14] activities. Copper(II) ions occur universally in living systems and are crucial for numerous metabolic and enzyme-mediated functions. Copper(II) complexes of benzimidazole possess medicinal properties and have been studied elaborately.[15,16] Research work on the biological activity of metal complexes of Schiff bases of 2-amino benzimidazole with salicylaldehyde and benzaldehyde has been explored.[17] Cancer is a deadly disease, and the rate of mortality declines due to cancer tissues. Cervical

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cancer in women was caused by the HeLa cell lines. Cervical cancer results in abnormal vaginal bleeding, pain during intercourse, pelvic pain, and unusual vaginal discharge. Cytotoxic activity of 1,3,5-substituted-2,3-dihydro-2-imino-benzimidazoles against HeLa cell lines was reported, and the hydrazone compound was found to be effective against the cells.[18] In this article, the role of the substituent in benzaldehyde on the biological activity is studied by pursuing studies on Schiff bases with 2-aminobenzimidazole. This work presents the synthesis, characterisation, and pharmacological investigation of 2-aminobenzimidazole-based Schiff bases and their corresponding Cu(II) complexes. 2-Aminobenzimidazole Schiff bases support key goals of the United Nations Sustainable Development Goals by aiding drug development and helping fight disease (as highlighted by the World Health Organisation), while also contributing to water purification and greener chemical processes.

EXPERIMENTAL

Materials

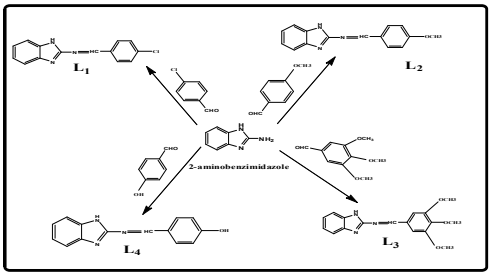
2-aminobenzimidazole, p-chloro benzaldehyde, p-methoxy benzaldehyde, 3,4,5-trimethoxy benzaldehyde, p-hydroxy benzaldehyde, $CuCl_2$. $2H_2O$, Diphenyl Picryl Hydrazyl radical (DPPH)were acquired from Sigma Aldrich.

Methods

The Schiff bases and their Cu(II) complexes were analysed for elements at SAIF, CDRI, Lucknow. Magnetic moments were measured with a Sherwood Mark 1 balance, molar conductance was recorded in DMSO using an ELICO CM-180 meter, and ESI mass spectra were obtained on a JEOL-GC MATE-2 instrument at IIT Madras. UV–Visible spectra were obtained using a Shimadzu UV-1800 spectrophotometer. IR spectra were recorded from KBr pellets on a Shimadzu FT-IR (IR Affinity-1) instrument. NMR spectra were collected on a Bruker DRX-300 MHz spectrometer using $CDCl_2$ as the solvent and TMS as the internal reference. BSA and DNA binding were studied using electronic absorption spectroscopy. Antimicrobial activity was tested by the well-diffusion method, and the anticancer activity of the ligands and their Cu(II) complexes was evaluated against HeLa cells.

Synthesis of Ligand Molecules

The ligands (L1–L4) were synthesised by the reaction of 2-aminobenzimidazole (1.99 g, 15 mmol) with substituted benzaldehydes, namely p-chloro benzaldehyde (1.99 g, 15 mmol), p-methoxy benzaldehyde (1.99 g, 15 mmol), 3,4,5-trimethoxy benzaldehyde (2.10 g, 15 mmol), and p-hydroxybenzaldehyde (1.99 g, 15 mmol). Each reaction was performed in ethanol with an equal molar ratio (1:1) and stirred for 3 hours. The reaction progress was observed using TLC, and upon completion, the formed colored precipitates were collected by filtration, washed systematically with ethanol, and dried under vacuum.



Scheme-1: Synthesis of Schiff Base Ligands

Ligand-1: Yield 75%. *Anal.* (%) Calcd. for $C_{14}H_{10}N_3Cl$: C, 65.88; H, 3.92; N, 16.47; Cl, 13.72 Found: C, 65.63; H, 3.85; N, 16.38; Cl; 13.65 ^{1}H-NMR (400 MHz, DMSO, δ, ppm): 9.46(s, -CH=N) 6.14 -8.10 (m, aromatic ring protons), IR (KBr, cm^{-1}) 1645 ν(-CH=N), 3349 ν(N–H). UV–vis ((Ethanol, λ_{max}, nm): 332 nm; 409 nm.

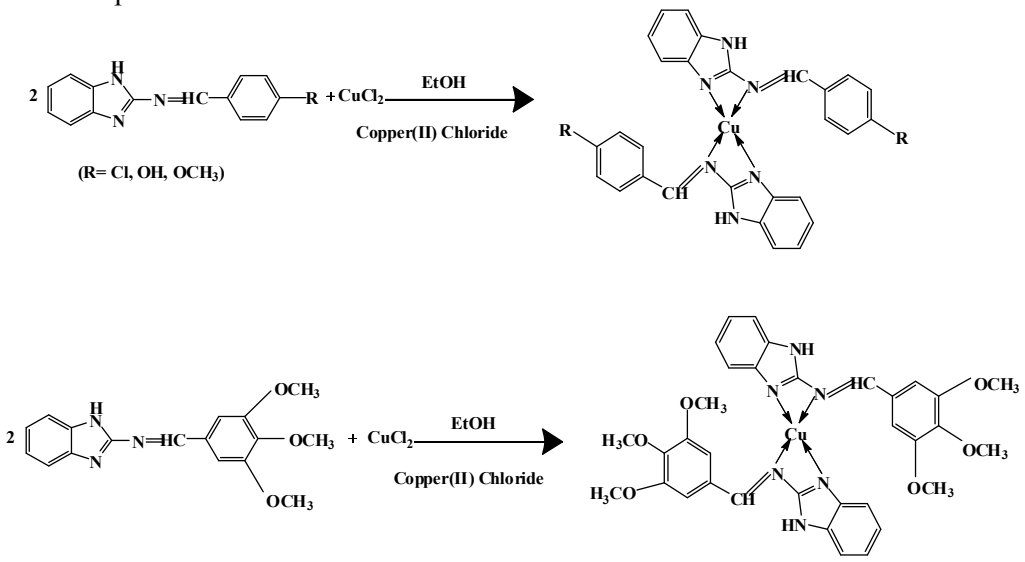
Ligand-2: Yield 78%. *Anal.* (%) Calcd. for $C_{15}H_{13}N_3O$: C, 71.72; H, 5.17; N, 16.73; Found: C, 71.62; H, 5.09; N, 16.65; ^{1}H-NMR (400 MHz, DMSO, δ, ppm):9.37 (s, -CH=N) 6.12- 7.57 (m, aromatic ring protons), IR (KBr, cm^{-1}) 1633 ν(-CH=N), 3362 ν(N–H). UV–vis ((Ethanol, λ_{max}, nm): 328 nm; 407 nm.

Ligand-3: Yield 72%. *Anal.* (%) Calcd. for $C_{17}H_{17}N_3O_3$: C, 65.59; H, 5.46; N, 13.52; Found: C, 65.45; H, 5.23; N, 13.44; ^{1}H-NMR (400 MHz, DMSO, δ, ppm):9.4 (s, -CH=N) 6.09 -7.57 (m, aromatic ring protons), IR (KBr, cm^{-1}) 1624 ν(-CH=N), 3540 ν(N–H). UV–vis ((Ethanol, λ_{max}, nm): 330 nm; 410 nm.

Ligand-4: Yield 74%. *Anal.* (%) Calcd. for $C_{14}H_{11}N_3O$: C, 70.88; H, 4.64; N, 17.72; Found: C, 70.65; H, 4.42; N, 17.53; ^{1}H-NMR (400 MHz, DMSO, δ, ppm): 9.79 (s, -CH=N) 6.19 -7.91 (m, aromatic ring protons). IR (KBr, cm^{-1}) 1638 ν(-CH=N), 3362 ν(N–H). UV–vis ((Ethanol, λ_{max}, nm): 334 nm; 406 nm.

Formation of Cu(II) Complexes

The Cu(II) complexes were synthesised by mixing $CuCl_2$ (0.34 g, 2 mmol) with the Schiff base ligands—L1 (1.02 g, 4 mmol), L2 (1.004 g, 4 mmol), L3 (1.244 g, 4 mmol), and L4 (0.94 g, 4 mmol)—in ethanol using a 2:1 molar ratio. Each reaction mixture was stirred for 3 hours, after which the precipitated products were collected by filtration, washed repeatedly with ethanol, and dried under vacuum. The synthetic route is presented in Scheme-2.



Scheme-2: Synthesis of Copper (II) Complexes

L1-Cu (II) Complex: Yield 60%. *Anal.* (%) Calc. for $C_{28}H_{20}N_6CuCl_4$: C, 52.25; H, 3.11; N, 13.06; Cl, 21.77; Cu, 9.79. Found: C, 52.12; H, 3.01; N, 12.95; Cl,21.65; Cu,9.65. Λ_{m-} 47 S m^2 mol^{-1}; IR (KBr, cm^{-1}) 1557 ν(C=N), 515 (M–N), UV–vis (DMSO, λ_{max}, nm): 335 nm and 578 nm.

L2-Cu(II) Complex: Yield: 55%. *Anal.* (%) Calcd for $C_{30}H_{26}N_6O_2CuCl_2$: C, 56.69; H, 4.09; N, 13.22; Cl, 11.02; Cu, 9.92. Found: C, 56.42; H, 3.98; N, 13.12; Cl, 11.02; Cu, 9.85. Λ_{m-} 50 S m^2 mol^{-1}; IR (KBr, cm^{-1}) 1568 ν(CH=N), 520(M–N). UV–vis (DMSO, λ_{max}, nm):338 nm and 575 nm.

L3- Cu(II)Complex: Yield: 53%. *Anal.* (%) Calcd for $C_{34}H_{34}N_6O_6CuCl_2$: C, 54.03; H, 4.50; N, 11.12; Cl, 9.27; Cu, 8.34. Found: C, 53.98; H, 4.28; N, 11.02; Cl, 9.12; Cu, 8.21. Λ_{m-} 48 S m^2 mol^{-1}; IR (KBr, cm^{-1}) 1575 ν (CH=N), 551 (M–N), UV–vis (DMSO, λ_{max}, nm): 345 nm and 568 nm.

L4- Cu(II) Complex: Yield: 53%. *Anal.* (%) Calcd for $C_{28}H_{22}N_6O_2CuCl_2$: C, 55.35; H, 3.62; N, 1.32; Cl, 11.53; Cu, 10.37. Found: C, 55.28; H, 3.58; N, 1.22; Cl, 11.48; Cu, 10.25. Λ_{m-} 52 S m^2 mol^{-1}; IR (KBr, cm^{-1}) 1579 ν (CH=N), 512 (M–N), UV–vis (DMSO, λ_{max}, nm): 359 nm and 562 nm.

Antioxidant Activity

The antioxidant properties of the ligands and their Cu(II) complexes were evaluated by using the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The percentage inhibition was intended using the equation:

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0 \ ] \times 100,$$

Where A_0 denotes the absorbance of the control, and A_1 corresponds to the absorbance of the test sample.[19]

Anticancer Studies

Effect of Schiff Bases and their Corresponding Metal Complexes on the Viability and Proliferation of HeLa Cell WWW21`a2Lines

The biocompatibility of the test compounds was examined by an *in vitro* MTT (3(-[4,5-dimethylthiazol-2-yl]-2,5diphenyl tetrazolium bromide) assay performed on cervical cancer cell lines (HeLa) to evaluate cell viability. HeLa cells were established and then distributed into 96-well plates at a density of 2x 103 cells /ml, with each well receiving 100µl of suspension. HeLa cells were exposed to a range of Schiff bases and their Cu(II) complexes concentrations from 10 to 50µg/ml and then incubated as needed. After the incubation period, MTT solution was added to the cells and incubated at a temperature of 37ºC for three hours. The purple formazan crystals were dissolved in 100µl of dimethyl sulfoxide (DMSO). Optical density (OD) was evaluated at a wavelength of 570 nm.

$$\text{Hela cell viability \%} = \left(\frac{\text{OD of treated sample}}{\text{OD of untreated sample}}\right) \text{ X } 100$$

Identification of Cellular Mortality by Dual Staining Technique

A dual staining technique was employed (acridine orange (AO) and ethidium bromide (EB)) to identify the dead cells in cultured cervical cancer cells. The cells were maintained in 6-well plates at a constant temperature of 37º C for 24 h, with varying concentrations of Schiff bases and their Cu(II) complexes at 10, 20, and 40 µg/ml. After incubation, the cells were treated with acridine orange and ethidium bromide for 30 minutes. The cells were then analysed using a fluorescence microscope to detect cell death.

Assessment of Reactive Oxygen Species (ROS) Generation in HeLa Cells

Assessment of intracellular reactive oxygen species accumulation was performed using the DCFH-DA (2',7'-dichlorodihydrofluorescein diacetate) fluorescent staining technique. HeLa cells were cultured and exposed to a range of test compound concentrations: 10, 20 and 40 µM. The cells were placed in a CO_2 incubator at 37º C for 24 h. After the incubation period, the cells were stained with DCFH-DA for 15 min. Fluorescence intensity was subsequently analysed using a fluorescent microscope at 530 nm.

RESULTS AND DISCUSSION

Copper (II) complexes prepared from 2-aminobenzimidazole appended Schiff base ligands, and the brief synthetic protocol was discussed in the experimental section. The proposed structure of Schiff bases and copper (II) complexes was analysed by elemental analysis, diverse spectroscopic (electronic, FT-IR, ^{1}H-NMR and Mass) techniques. The ligands were soluble in chloroform, ethanol, methanol, acetone, acetonitrile, DMSO and DMF. The Cu(II) complexes were soluble in DMSO and DMF.

Interpretation of FT-IR Spectra

FT-IR spectral analysis of the Schiff base ligands (L1–L4) and their Cu(II) complexes was carried out, and the results are summarised in Table-1. The IR spectra of ligands (L1-L4) show a band at 1645 cm^{-1}, 1633 cm^{-1}, 1624 cm^{-1}, and 1638 $cm^{-1,}$ respectively, attributed to azomethine stretching frequency. Also, the Schiff bases exhibit peaks at 1540 cm^{-1}, 1547 cm^{-1}, 1550 cm^{-1}, 1542 $cm^{-1,}$ respectively, which relate to ν(C=N) of the imidazole ring. The lowering of the band ν(C=N) benzimidazole values (1530-1538 cm^{-1}) in metal (II) complexes suggests the coordination of the ring nitrogen to the metal (II) ion.[14] In the bands of the copper(II) complexes (L1Cu-L4Cu), the azomethine peaks shift to lower frequencies at 1610-1625 cm-1, indicating the chelation of azomethine nitrogen with the metal (II) ions.[20] A low intensity band observed between 512-556 cm^{-1} is associated with (M-N) vibrations, inferring metal-nitrogen coordination.[21]

Table-1: FT-IR Data of Ligands and Copper(II) Complexes (cm^{-1})

Compounds	υ(-CH=N)	ν(C=N) imidazole	υ (M-N)
L1	1645	1540	-
L2	1633	1547	-
L3	1624	1550	-
L4	1638	1542	-
L1-Cu	1625	1530	515
L2-Cu	1620	1535	520
L3-Cu	1610	1538	551
L4-Cu	1625	1530	512

Analysis of UV-Visible Spectra

The ligands (L1–L4) exhibit characteristic electronic absorption bands at 332 nm (30120 cm^{-1}) and 409 nm (24449 cm^{-1}) for L1; 328 nm (30487 cm^{-1}) and 407 nm for L2; 330 nm (30303 cm^{-1}) and 410 nm (24390 cm^{-1}) for L3; and 334 nm (29940 cm^{-1}) and 406 nm (24630 cm^{-1}) for L4, which are attributed to $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transitions. The UV–Visible spectra of the Cu(II) complexes were recorded in DMSO. The Cu(II) complexes (L1–Cu to L4–Cu) display absorption bands at 509 nm (19646 cm^{-1}), 520 nm (19230 cm^{-1}), 532 nm (18796 cm^{-1}), and 565 nm (17699 cm^{-1}), corresponding to d–d electronic transitions characteristic of square-planar geometry. The nature of the electronic transitions suggests that the complexes are square-planar, which is supported by the measured magnetic moment of 1.75 B.M. for the Cu(II) complexes. Therefore, both the UV–Visible data and magnetic measurements propose the square-planar nature of the complexes.[22] The spectral data are presented in the supplementary file Table-S1.

NMR Spectral Analysis

Table-2 depicts the ^1H NMR spectra of the Schiff bases (L1–L4). Each ligand shows a characteristic singlet for the azomethine (–CH=N–) proton at 9.46, 9.37, 9.40, and 9.79 δ ppm, respectively. The aromatic protons appear as multiplets within the ranges 6.14–8.10 ppm for L1, 6.12–7.57 ppm for L2, 6.09–7.57 ppm for L3, and 6.19–7.91 ppm for L4. Additionally, ligands L2 and L3 display extra singlet peaks at 3.87 and 3.34 δ ppm, corresponding to methoxy groups attached to the aromatic ring. The ^1H NMR spectra are presented in Fig.-S2(a–d).

Table-2: NMR Characterisation of Schiff Base Ligands (L1-L4)

Sample	Azomethine proton(ppm)	Aromatic proton(ppm)	Methoxy proton(ppm)
L1	9.46	6.14-8.10	-
L2	9.37	6.12-7.57	3.87
L3	9.40	6.09-7.57	3.34
L4	9.79	6.19- 7.91	-

ESI-Mass

The mass spectra of the ligands (L1–L4) exhibited molecular ion peaks at m/z 255, 251, 311, and 237, respectively. For their Cu(II) complexes, molecular ion peaks were observed at m/z 643, 635, 755, and 607, confirming the species $[ML]Cl_2$. These spectral data confirm the formation of mononuclear transition metal complexes. Fragmentation further reveals the loss of chloride ions, indicating that the chlorine atoms are located outside the coordination sphere. The respective spectra are provided in Fig.-S3(a-d).

Antioxidant Activity

The antioxidant properties of the ligands and their Cu(II) complexes were assessed using the DPPH free radical assay. UV–Vis spectral analysis showed that the characteristic DPPH peak gradually decreased in intensity with increasing concentration of the Schiff bases and Copper(II) complexes. The data obtained at different micromolar concentrations of the Schiff bases and their complexes are summarised in Table-3. The antioxidant efficiency improves as electron/proton donation is promoted, leading to a colour shift in the DPPH solution from purple to yellow. Among the tested compounds, ligand L3 and its complex L3–

Cu show the highest percentage of inhibition, this may be attributed to the electron-donating methoxy group. The scavenging activity of the Copper(II) complexes follows the sequence: L3–Cu > L4–Cu > L2–Cu > L1–Cu.[23] Figure-S4(a,b) shows the percentage inhibition of the DPPH radical by the Schiff bases and their Copper(II) complexes.

Table-3: Antioxidant Activities of Ligands and their Copper(II) Complexes

Sample	10 µmol	20 µmol	30 µmol	40 µmol	50 µmol
L1	5.84	8.35	8.56	12.30	19.72
L2	3.9	14.48	18.68	23.17	24.54
L3	19.73	26.05	28.53	29.9	35.55
L4	12.76	18.38	19.81	23.7	25.92
L1-Cu	0.72	2.36	4.1	5.5	9.2
L2-Cu	1.7	4.1	5.9	10.11	12.3
L3-Cu	11.93	12.93	16.88	20.20	22.34
L4-Cu	5.6	8.2	10	12	15

Anticancer Studies

The cytotoxic effects of Schiff bases and their corresponding metal complexes on HeLa cells were evaluated using the MTT assay, which indicated a significant reduction in cell viability depending on the dosage, with higher doses showing increased toxicity (Fig.-2). Morphological examination of HeLa cells exposed to the test compounds showed significant differences in cell morphology, growth and structural integrity compared to the untreated controls. Treated cells showed significant morphological changes such as irregular shapes, shrinkage and detachment, which are indicative of cytotoxic stress caused by the test compounds. The results of the cytotoxic assay showed that the Schiff bases and their corresponding metal complexes exhibited increased cytotoxic effect after 24 hours, demonstrating a concentration-dependent relationship with the IC_{50} values of 60.15 µM, 40.31 µM, 40.33 µM, 40.27 µM for L1, L2, L3, and L4, respectively (Table-4). It is evident from the results that L1 showed maximum cytotoxicity against HeLa cells. Similarly, the L1-Cu complex showed a high IC_{50} value of 60.20 µM. Conversely, the least cytotoxicity, 19.88 µM, was observed for the L4-Cu complex.[24]

Table-4: Cytotoxic Evaluation of Ligands and Cu(II) Complexes

Schiff bases	IC_{50} (µM)	Complexes	IC_{50} (µM)
L1	60.15	L1-Cu	60.20
L2	40.31	L2-Cu	40.15
L3	40.33	L3-Cu	39.96
L4	40.27	L4-Cu	19.88

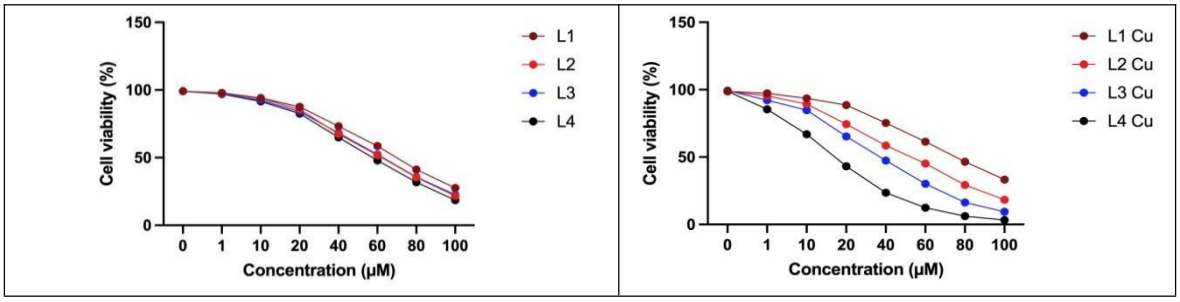


Fig.-1: Cytotoxic Effects of Free Ligands and their Copper(II) Complexes against HeLa Cells

The degree of apoptosis in HeLa cells treated with the test compounds was evaluated using AO/EtBr staining. The microscopic images shown in Fig.-3 illustrate the results of the double labelling. The untreated control cells showed green fluorescence, as indicated by AO/EtBr staining, whereas the HeLa cells supplemented with Schiff bases and their corresponding metal complexes showed striking orange

fluorescence due to EtBr staining. It is evident from the results that both L and L-Cu derivatives induced early apoptosis at 20 µM and late apoptosis and necrotic stages at 40 µM. The data presented in Fig.-6 confirms that the Schiff bases and their corresponding metal complexes treatment resulted in a significant increase in ROS production in HeLa cells. Cells exposed to different concentrations of Schiff bases (L1, L2, L3, and L4), particularly 20 and 40 µM, showed a significant increase in ROS, which was clearly illustrated by intense green fluorescence. Conversely, untreated control cells revealed bright green fluorescence at all concentrations tested. These results indicate that the L-Cu complex is more effective in ROS formation than L1, L2, L3 and L4, respectively. The increased accumulation of ROS may be due to the triggering of apoptotic pathways within the cellular environment. The significant green fluorescence observed in the treated groups highlights the ability of the test compounds to significantly increase ROS production in HeLa cells, thereby triggering oxidative stress and subsequent apoptosis. The connection between ROS and cellular toxicity is crucial for understanding the mechanisms by which Schiff bases exert their deleterious effects. Elevated ROS levels can disrupt cellular homeostasis and lead to cell death.[25] This phenomenon highlights the importance of studying oxidative stress as a key factor in Schiff base-mediated toxicity, particularly in HeLa cells. It is evident from the above findings that an increase in the ROS production conferred by Schiff's bases and their corresponding metal complex is likely to be a significant contributor to the oxidative stress observed in HeLa cells.

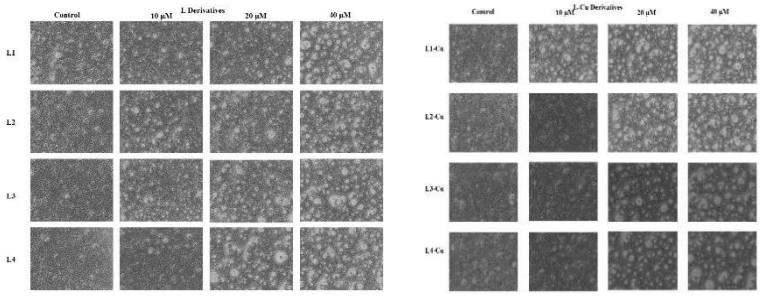


Fig.-2: Cytotoxicity of Schiff Bases and Copper(II) Complexes Against HeLa Cell Lines

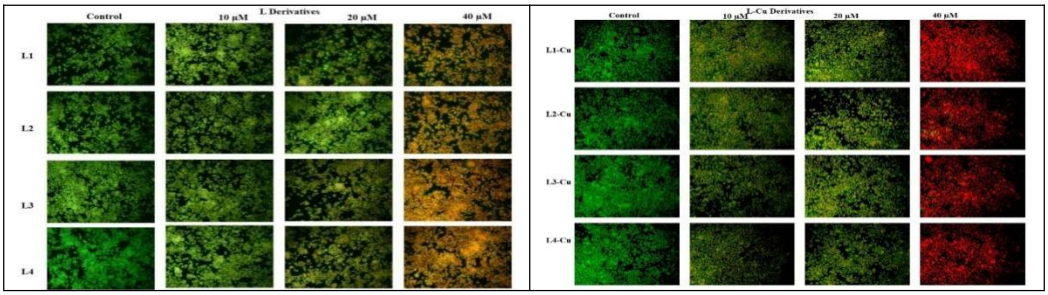


Fig.-3: Apoptotic Effect of Schiff Bases and Copper(II) Complexes on HeLa Cell Lines AO/EtBr Dual Staining

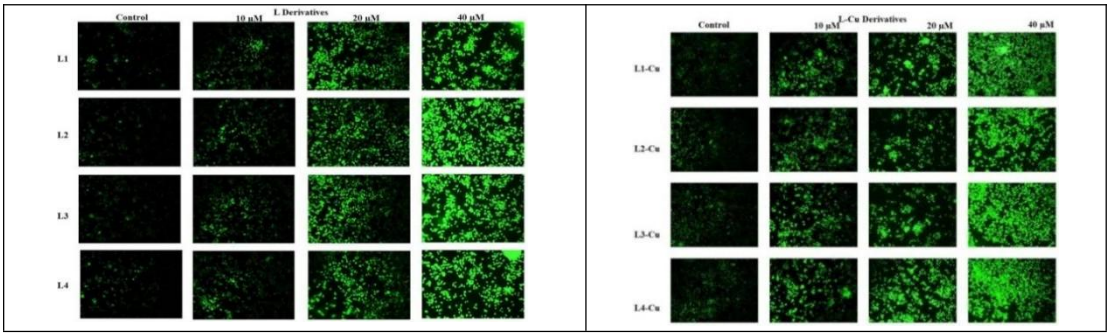


Fig.-4: Effect of Schiff Bases and Copper(II) Complexes on Reactive Oxygen Species Production on HeLa Cell Lines

The cytotoxic potential of the Schiff bases and their Copper(II) complexes toward human cervical cancer (HeLa) cells was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The IC_{50} values for the synthesised compounds are provided in Table-4. These values were determined from the plots showing the percentage of cell viability versus compound concentration. As shown in Fig.-3 the survival rate of cancer cells gradually decreases with an increase in complex concentration. This result confirms the inhibitory efficiency of the synthesised compounds and their strong binding affinity toward cervical cancer cells. The cytotoxic effect of the Schiff bases followed the order L4 > L3 > L2 > L1, indicating that ligand L4 exhibits the highest cytotoxic potential among the series. Likewise, among the Cu(II) complexes, L4–Cu displayed the strongest cytotoxicity toward the cancer cell lines relative to the others. In summary, both L4 and L4–Cu emerged as the most active compounds, indicating notable anticancer potential. The fluorescence images of HeLa cells exposed to the ligands and Cu(II) complexes are presented in Fig.-2 to 4. M. Sankarganesh *et al.* synthesised a new Schiff base from 2-hydroxy-3-methoxybenzaldehyde and 2-amino-4,6-dimethoxypyrimidine. The ligand L4 in the present study has the IC50 value of 40.27 µM compared to the already reported value (98.2 µM) for HeLa cells, demonstrating its higher cytotoxic potency.[26] Afsal Hussain *et al.* investigated transition metal complexes of a benzimidazole-derived scaffold as potential anticancer agents, using 1-methyl-2-aminobenzimidazole and 2-hydroxynaphthaldehyde. The copper complex in their study displayed an IC_{50} value of 23 µM, indicating the greater potency of the present L4 -Cu complex (19.88 µM).[27] 2-amino benzimidazole contributes to SDG-3 (Good Health and Well-being) through its anticancer activity by supporting the development of improved therapies. It also aligns with SDG-9 (Industry, Innovation and Infrastructure) by advancing drug discovery and with SDG-12 (Responsible Consumption and Production) through greener synthesis methods.[28]

CONCLUSION

Schiff bases and their Cu(II) complexes were investigated and shown to act as bidentate ligands, coordinating through azomethine and imidazole nitrogen atoms to form mainly square planar geometries. Antioxidant studies using the DPPH assay revealed that methoxy-substituted compounds exhibit superior radical scavenging activity, while cytotoxicity tests on HeLa cells indicated that hydroxyl-substituted derivatives possess enhanced anticancer effects. Overall, 2-aminobenzimidazole-based ligands and their Cu(II) complexes show promising antioxidant and anticancer potential, contributing to sustainable development goals such as good health and well-being (SDG 3) and responsible consumption and production (SDG 12) by promoting the development of efficient and potentially safer therapeutic agents.

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AUTHOR CONTRIBUTIONS

The present work is related to *SDG-9:Industry, Innovation, and Infrastructure*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

A.Krishnaveni https://orcid.org/0009-0008-3330-7208
M. Kalanithi https://orcid.org/0000-0002-8734-4548
M.Karthikeyan https://orcid.org/0000-0003-2208-3487
U.Deenathayalan https://orcid.org/0009-0007-7116-6250

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