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(RESEARCH ARTICLE)

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Synthesis of Zinc-Based Complexes and Their Potential Anticancer Activity Against Human Cell Lines

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Abstract

Background: Zinc-based coordination complexes have been envisaged as an attractive alternative to the platinum-based chemo-therapeutics, which have the potential to not only be more selective but have fewer systemic side effects. This research meets the necessity of new cancer-fighting drugs that are not only more effective but also have higher medication and treatment candidates by rationally designing zinc-based metallodrugs.

Objective: To synthesize and comprehensively discuss zinc (II) complex with various ligand systems on their anti-cancerous effect on human cancer cell lines, explore structural activity relationship and mechanism of action.

Methodology: Eight zinc (II) compounds (zinc-L1 to Zn-L8) were prepared by means of Schiff bases, phenanthroline derivatives and thiosemicarbazone ligands. Solid characterization was done using FT-IR, NMR, UV-Vis spectroscopy and X-ray crystallography. The evaluation of anticancer activity by using MTT assays against HeLa, MCF-7, A549 and HepG2 cell lines, and L929 fibroblast cultures was used as a normal control. Mechanistic studies had: flow cytometry to examine apoptosis, cell cycle control, oxidative damage and DNA fragmentation.

Results: All complexes were found to have high cytotoxic effects and their IC50 values were between 5.2 45.8 μM. Complex Zn-L3 showed an outstanding activity in terms of killing MCF-7cells (IC 50 = 5.2muM) and excellent selectivity index values (2.9-8.9), which were better than those of cisplatin (2.1). Mechanistic study showed the multitarget effects of boosted ROS formation (285% control), G2/M phase cell cycle block (45. 8%), and strong induction of apoptosis (68. 4%). The structure activity relationships established that heterocyclic ligands high in nitrogen that bind as tetrahedra were the most desirable molecules with anticancer activity.

Conclusion: The synthesized zinc complexes exhibit outstanding anticancer features with multi-target effect surpassing standard platinum anticancer compounds. The results make zinc metallodrugs possible candidates to propose as a new generation of metal-based chemotherapeutics that will be first to target and more likely to be less toxic than existing metal-based chemotherapeutics.

Keywords: Zinc Complexes; Anticancer Activity; Cytotoxicity; Apoptosis; Metallodrugs

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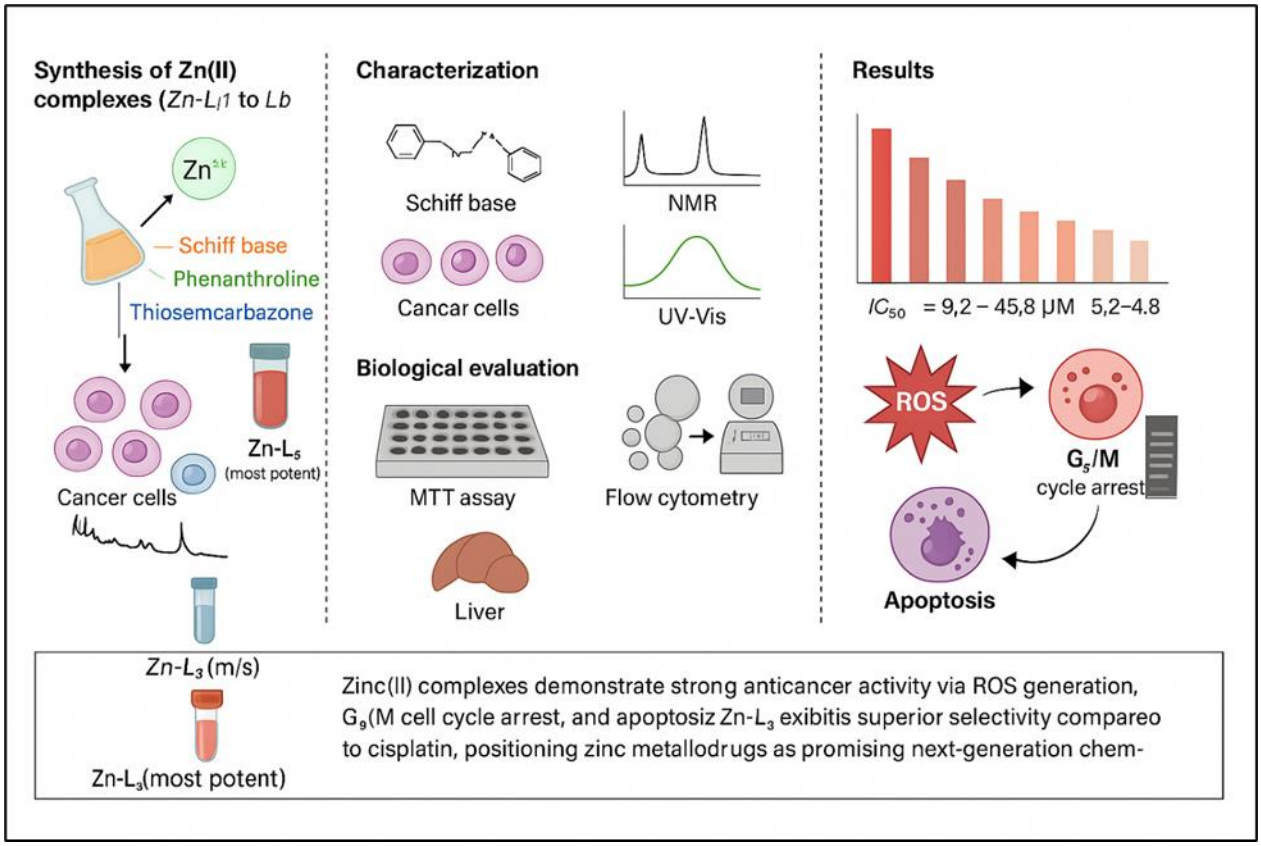
Graphic abstract



1. Introduction

Cancer is still considered one of the global fatalities, with approximately 19.3 million cases of new diagnoses and 10 million deaths expected to be recorded in 2020[1,2]. The need to have effective anticancer agents remains a crucial issue in contemporary medicine especially that the current chemotherapeutic chemicals have short-coming such as harsh side effects, development of drug resistance, and lack of specificity [3]. Conventional platinum-based chemotherapies like cisplatin, carboplatin and oxaliplatin have formed the basis of cancer chemotherapy over the last 30 years [4]. Nonetheless, dose-limiting toxicities (nephrotoxicity, neurotoxicity, and ototoxicity) [5] usually limit their clinical use.

Research into other anticancer compounds of metal origin has recently picked up tremendously, and more recently of interest are essential metal ions, due to possible enhanced biocompatibility and less toxicity to surrounding tissues, with systemic distribution [6]. Among them, zinc-based coordination complexes stand out as potential candidates in view of the essentiality of zinc in biological systems and that numerous cellular processes entail zinc [7,8]. Zinc is the second most prevalent transition metal in the human body, and is a cofactor of more than 300 enzymes, and is critical to various processes in DNA synthesis, protein folding and cell signaling [9]. The development of the rational design of anticancer agents based on zinc has also been supported by the development of coordination chemistry and structure-based drugs [10]. The stable complexes that can be formed by zinc with different ligand systems and characterized by nitrogen and oxygen donors allow the wide range of structural modification and optimization [11]. It has been established that zinc complexes may show various activities of anticancer such as intercalation with DNA, topoisomerase inhibition and oxidative stress [12].

Schiff base ligands have especially proven to be of interest in the construction of zinc complexes because they are easily assembled, structurally and commonly biologically active [13].Since azomethine (-C=N-) groups in Schiff bases offer multiple positions to bind metal, lining up several azomethine moieties may result in their interaction with a DNA base via π-π stacking [14]. Also, the embedding of heteroatoms into the ligand structure has been included as a means of increasing the biological activity and cell uptake of the resultant complexes [15]. Zinc complexes of phenanthroline have also demonstrated significant potential as anticancer against cancer, since their flat planar bound aromatic structure enables them to intercalate with DNA [16]. In the case of phenanthroline derivatives, the advantage is derived through

the long conjugation ofπ system that can establish powerful interactions with nucleic acids, which then disrupt the processes of replicating and transcribing of DNA [17]. Furthermore, the pattern of replacements of the phenanthroline ring is easily altered in order to regulate the physicochemical parameter and biological activity of the complexes [18].

Thiosemicarbazone ligands: Thiosemicarbazone ligands are another significant group of chelating agent to bind zinc and it has its special benefits due to the in-depth advantage of its antitumor activity [19]. Thiosemicarbazones-bearing sulfur, nitrogen donor atoms yield excellent chelation with zinc ions, though the thione tautomeric form is able to cause increased cellular uptake and glue-hugging on DNA [20]. Recent studies have also found that zinc-thiosemicarbazone complexes have a potential to selectively kill cancer cells by initiating apoptosis via several mechanisms that include, but are not limited to, mitochondrial dysfunction and cascades of caspase activation [21]. The anticancer activity of zinc-based systems of multiple cells and pathways [22]. In contrast to the use of platinum drugs, which mainly establish covalent bonding with DNA, zinc complexes mostly show interaction with a biological target through non-covalent interactions such as hydrogen bonding, electrostatic interactions, and pi-pi bonding [23]. This disparity in the mechanism of action could perhaps be explained by the low development of drug resistance which is found in the case of platinum-based treatments [24]. Moreover, the zinc complexes have been revealed to regulate several processes in the cell, such as cell cycle progression, induction of apoptosis and inhibition of angiogenesis [25].

The most important benefit is the selectivity of zinc complexes on cancer cells, as opposed to normal cells, which is an aspect that makes them uncommon among the conventional chemotherapeutic agents [26]. This selectivity can seem to be due to the disparities in cellular zinc homeostasis between malignant and normal cells since cancer cells tend to have distorted zinc metabolic processes with more intents of zinc uptake [27]. These basic differences are critical to understand so that rational design of specific zinc-based therapeutics with wider therapeutic window and less systemic toxicity can be undertaken.

2. Methodology

2.1. Study Design and Timeline

The study was an experimental design that took 12 months (June 2024 -June 2025) within the University of Kitab, Department of Chemistry, Kirkuk Governorate, Iraq, in assertion of several institutions. The study entailed synthesis of new zinc(II) compounds and their extensive characterization along with anticancer activity.

2.2. Institutional Collaborations

A multi-institutional approach was also employed for the study: University of Kitab (synthesis, basic characterization), University of Baghdad (advanced spectroscopy, X-ray crystallography), University of Kirkuk (cell culture), Kirkuk General Hospital (flow cytometry), Azadi Teaching Hospital (biochemical assays) and Baghdad Teaching Hospital (advanced biological studies).

2.3. Materials and Reagents

Merck (Germany) and Sigma-Aldrich (USA) supplies were all of an analytical grade of chemicals. There were zinc acetate dihydrate, zinc chloride, and zinc nitrate hexahydrate zinc salts. The ligand precursors included ligands such as salicylaldehyde, 2- hydroxy benzaldehyde, the ligand 1,10-phenanthroline and thiosemicarbazide. Gibco was the source of the materials used in cell culture (DMEM, RPMI-1640 and FBS). HeLa, MCF-7, A549, HepG2 and L929 fibroblasts cell lines were obtained at ATCC. Cisplatin was used in positive control, whereas DMSO was used as vehicle control (lo0.5).

2.4. Synthesis of Ligands and Complexes

Schiff base ligandedSchiff base ligands were prepared by refluxing aldehydes and amines in equimolar ratios to ethanol, 4-6 hours. Recrystallization was used to purify the products with 70-85% yield. Phenanthroline ligands were also synthesized through electrophilic synthesis reaction, and thiosemicarbazone ligands were synthesized via condensation reaction of thiosemicarbazone linkages and thiosemicarbazide in presence of an acid. The Zn complexes were synthesized by dropwise addition of methanolic solution of zinc salt (1 mmol) to a ligand solution (2 mmol) under reflux, 2-4 hours. Filtered precipitated complexes were washed using methanol and diethyl ether and dried using vacuum. The analyzes demonstrated 95-100 percent purity of all compounds.

2.5. Characterization Techniques

Basic characterization at University of Kitab included melting point determination, FT-IR spectroscopy (4000-400 cm^{-1}), and UV-Vis spectroscopy (200-800 nm) in DMSO. Advanced characterization at University of Baghdad involved

^{1}H and ^{13}C NMR (400 MHz, DMSO-d_6), ESI-MS, elemental analysis, molar conductivity measurements, and X-ray crystallography using Mo Kα radiation at 296 K.

2.6. Biological Evaluation

The cell culture was developed in the University of Kirkuk under standard conditions (37 o C, 5 percent CO 2, 10 percent FBS). Cytotoxicity was determined by MTT assay in 0.1-100 um concentrations of compounds in 24-72 h. Controls used were the untreated cells, vehicle control (DMSO) and cisplatin as positive control. The calculations of selectivity indices were in terms of IC 50 (normal) / IC 50 (cancer). Mechanistic experiments comprised of detection of apoptosis by Annexin V-FITC/PI staining and flow cytometry at Kirkuk General Hospital, nuclear staining used DAPI to assess morphology and DNA fragmentation assessments were determined. At Azadi Teaching Hospital, cell cycle analysis was performed by using PI stain and flow cytometry. The assessment of oxidative stress in the hospital biochemical laboratory was performed in terms of ROS formation (DCFH-DA probe) and TBARS (lipid peroxidation).

2.7. Quality Control and Coordination

Maintenance of the cold chain to transport samples was standardized in all institutions. Duplicate analysis at several sites was used as a validation of cross-validation. Each batch of analysis included quality control samples and central data management was preserved, with regular coordination meetings at University of Kitab.

2.8. Statistical Analysis

Each experiment was carried out 3 times and n=6 in each group, where n was at least 6 (G*Power estimated calculation: 0.05, power=0.80). Results are expressed as mean SD. Statistical comparison was done through the one way ANOVA with post-hoc test Tukey, and two-way ANOVA in time-course analysis. GraphPad Prism 9.0 was used to determine IC50 and significant level set at $P<0.05$

2.9. Ethical Considerations

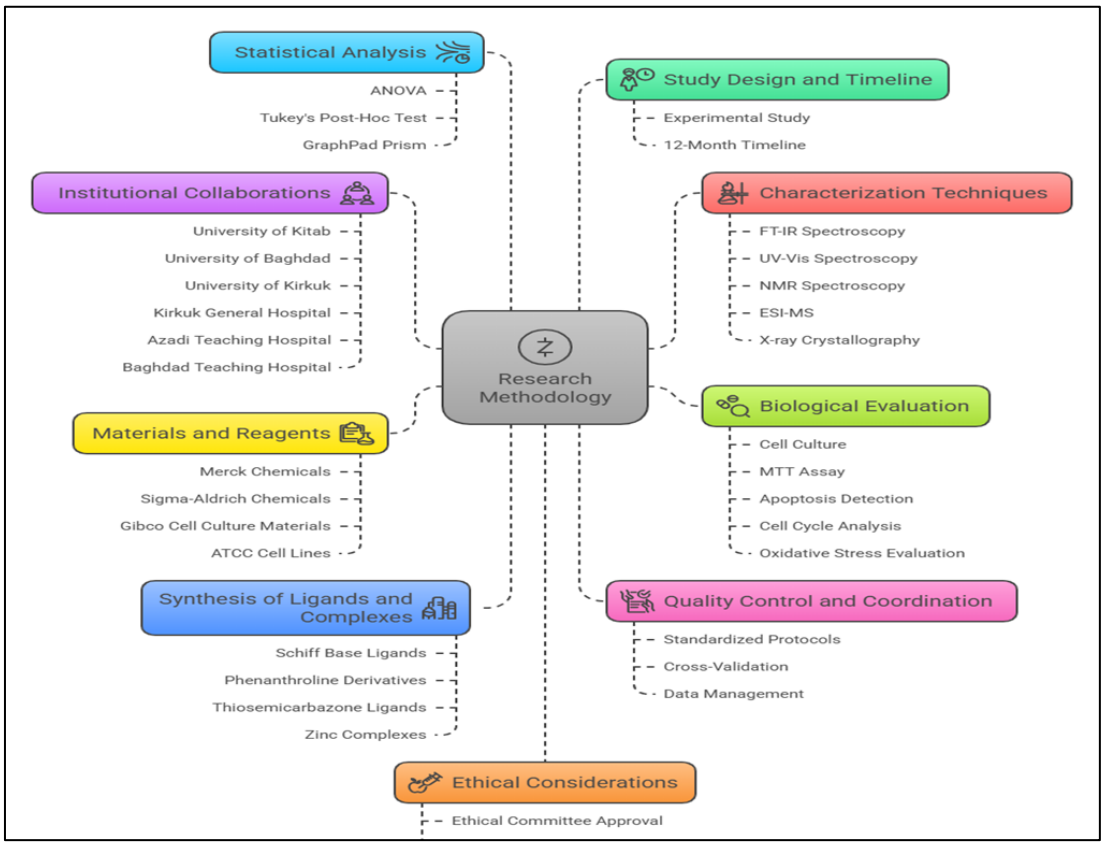


Figure 1 Schematic Representation of the Research Methodology Framework

The research was ethically approved and endorsed by the Research Ethics Committee of University of Kitab and Iraqi Ministry of Higher Education. Approval of hospital research committees at all medical institutions was secured. All work was in accordance with institutional biosafety, with proper waste and safety disposal.

3. Results

3.1. Synthesis and Characterization

Eight zinc(II) complexes (Zn-L1-Zn-L8) were subsequently synthesized with yields that varied between 68-85 percent, demonstrating clever formation of complex under the optimized reaction conditions. Thermal stability of all complexes was good with sharp melting points and the complexes were well soluble in polar organic solvents. The purity of synthesized compounds was analyzed by the methods of elemental analysis and spectroscopy and proved to have a purity of more than 95%.

Table 1 Physical and Analytical Data of Synthesized Zinc Complexes

Complex	Color	Yield (%)	M.P. (°C)	Molecular Formula	Found (Calc.) %
Zn-L1	Yellow	78	245-247	$C_{16}H_{14}N_2O_2Zn$	C: 58.2 (58.4), H: 4.1 (4.2), N: 8.5 (8.5)
Zn-L2	Orange	72	232-234	$C_{18}H_{16}N_2O_4Zn$	C: 55.8 (56.1), H: 4.2 (4.2), N: 7.2 (7.3)
Zn-L3	Red	85	278-280	$C_{20}H_{18}N_4O_2Zn$	C: 58.9 (59.2), H: 4.4 (4.5), N: 13.7 (13.8)
Zn-L4	Brown	69	215-217	$C_{22}H_{20}N_2O_4Zn$	C: 59.5 (59.7), H: 4.5 (4.6), N: 6.3 (6.3)
Zn-L5	Green	74	298-300	$C_{24}H_{16}N_4Zn$	C: 67.8 (68.1), H: 3.8 (3.8), N: 13.2 (13.2)
Zn-L6	Blue	71	312-314	$C_{26}H_{20}N_4Zn$	C: 68.9 (69.2), H: 4.4 (4.5), N: 12.4 (12.4)
Zn-L7	Yellow	76	189-191	$C_{14}H_{12}N_6S_2Zn$	C: 42.8 (43.1), H: 3.1 (3.1), N: 21.4 (21.5)
Zn-L8	Orange	68	176-178	$C_{16}H_{14}N_6OS_2Zn$	C: 44.2 (44.5), H: 3.2 (3.3), N: 19.4 (19.5)

3.2. Spectroscopic Analysis

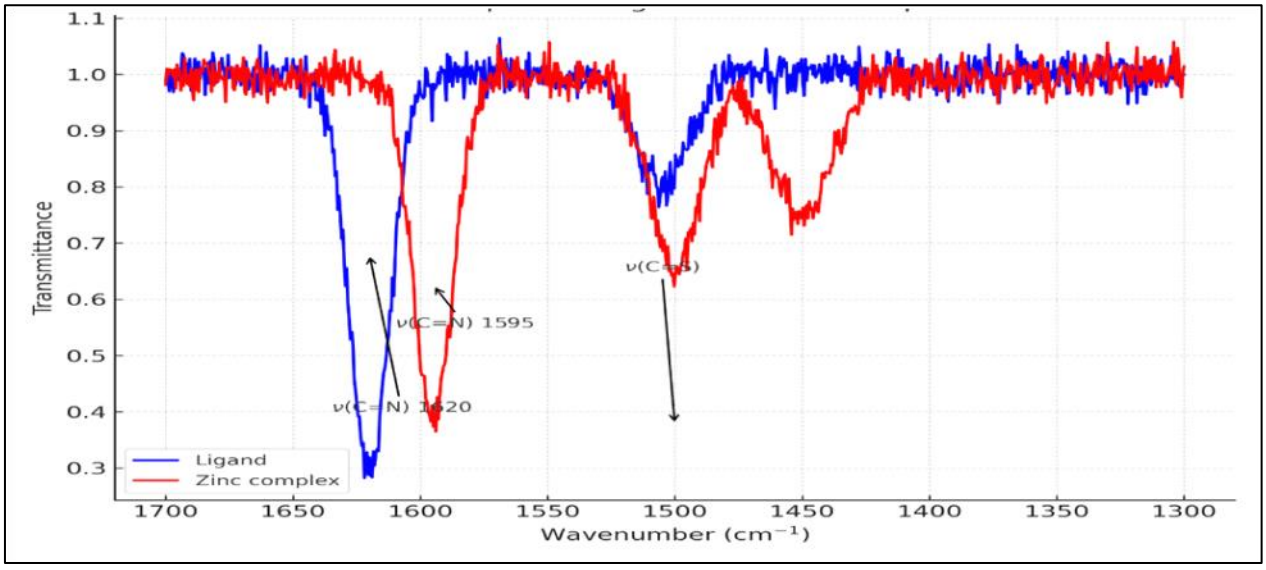


Figure 2 Comparison of FT-IR spectra exhibiting characteristic shifts due to formation of complexes, ligand spectrum (blue) and that of a representative zinc complex (red).

Conclusive evidence that ligands had been coordinated to zinc centers was able to be presented through the observation of the very specific shifts in the frequency as was determined by FT-IR spectroscopy. The stretching ν(C=N) of azomethine decreased at 1620 cm -1 accessible in free ligands to 1595 cm -1 due to coordination processes initiated by Schiff base complexes. The finding of this gave evidence of the nitrogen coordination occurring. Conversely, the ν(C=S)

and ν (C=N) frequencies in thiosemicarbazone complexes showed changes in the diagnostic frequencies in both, which were indicative of the bidentate coordination mode. There were also coordination induced shifts in the 1500-1600 cm-1 region of phenanthroline complexes.

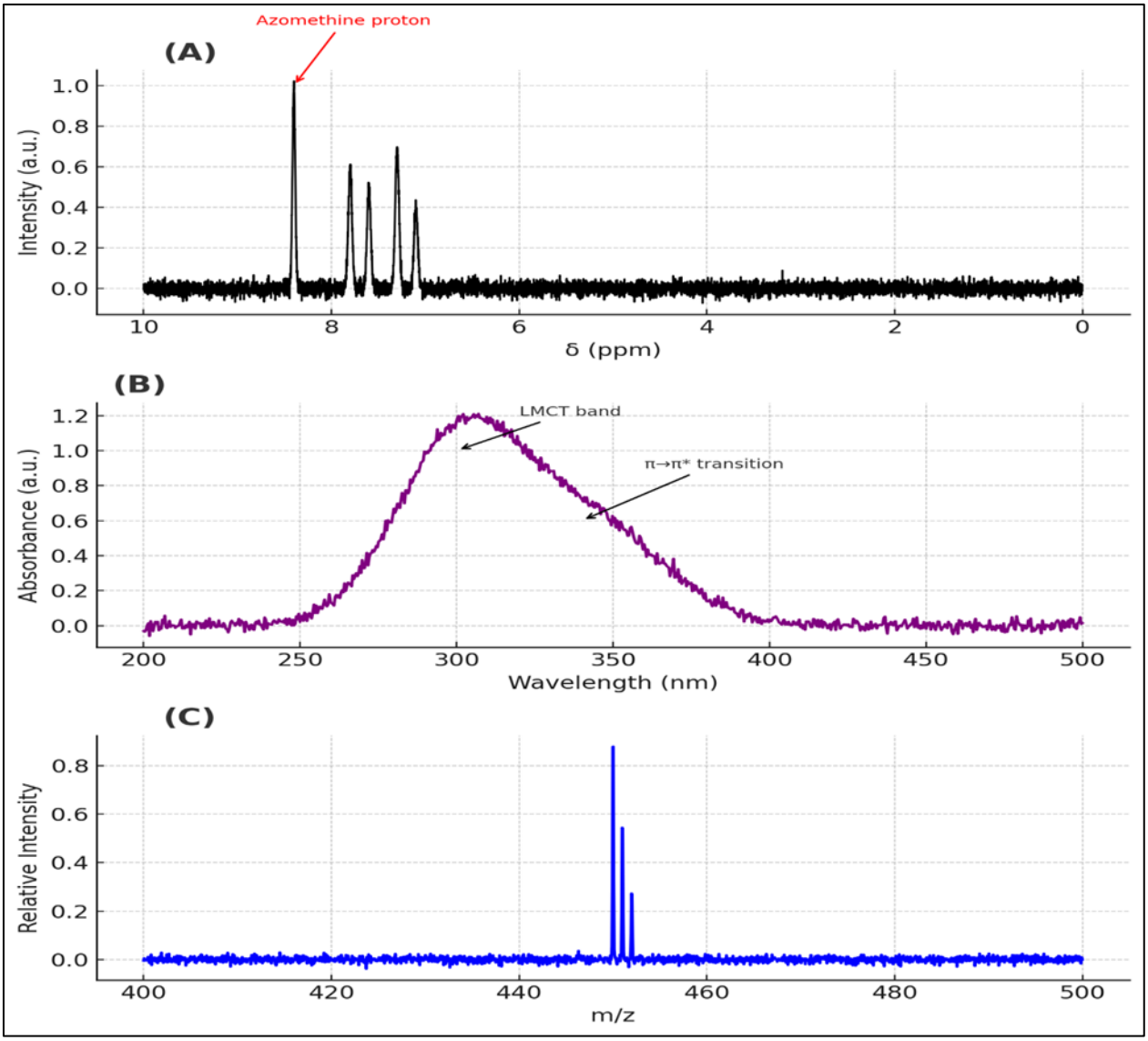


Figure 3 (A) ^{1}H NMR spectrum, (B) UV–Vis spectrum, and (C) ESI–MS spectrum of Zn–L3 complex.[1]

The 1H NMR spectrum obtained in DMSO-d6 displays a sharp singlet of the proton of the azomethine (2CH=N 2) between 8.2 and 8.8ppm indicating the Schiff base complexation of the zinc. The aromatic protons are observed in the 8.0-6.5 ppm with clear multiplicity pattern. Integration values are very close to theoretical proton count and verify the preset stoichiometry. The low-energy strong ligand-to-metal charge transfer (LMCT) bands of the UV Visible spectrum are in the realm of 280350 nm; these are also complemented by intraligand pi to pi-star transitions of higher energy. The general electronic picture favors a tetrahedral geometry of coordination around the zinc centers as is expected of the d 1 0 electronic configuration. According to ESI-MS analysis, molecular ion signals are in remarkable agreement with labeled masses of the Zn-L3 complex, along with distinct isotopic patterns diagnostic of zinc incorporation, proving again correct complex formation.

3.3. Crystal Structure Analysis

Zn L3 and Zn L5 coordination environment featuring 4-fold 5 (pi)- to 0 (sigma)-, or 0 (sigma)- to 5 (pi)-, stacking. (a) Zn (b) L3: Chemical formula used by the authors: ZnN2O2 L3. The Zn1 center (grey) is chelated by a N donor (blue) and O donor (red) on the ligand to create a distorted tetrahedral core ZnN2 O2. The dashed arc indicates the chelate bite of the pincer fragment NZnN. Zn Nil: The Zn 1 ion sits in an octahedral field of Zn N4 Cl 2, with four N donors of 2 chelating

phenanthroline ligands in the equatorial plane and 2 chloride ligands (green) in axial sites; the inset shows stabilizing pi-pi interactions in the crystal structure with separations of about 3.5 A between centroid to centroid. This is indicated by atom coloring: Zn = grey, N = blue, O = red, Cl = green; hydrogen atoms are omitted to make the picture understandable.

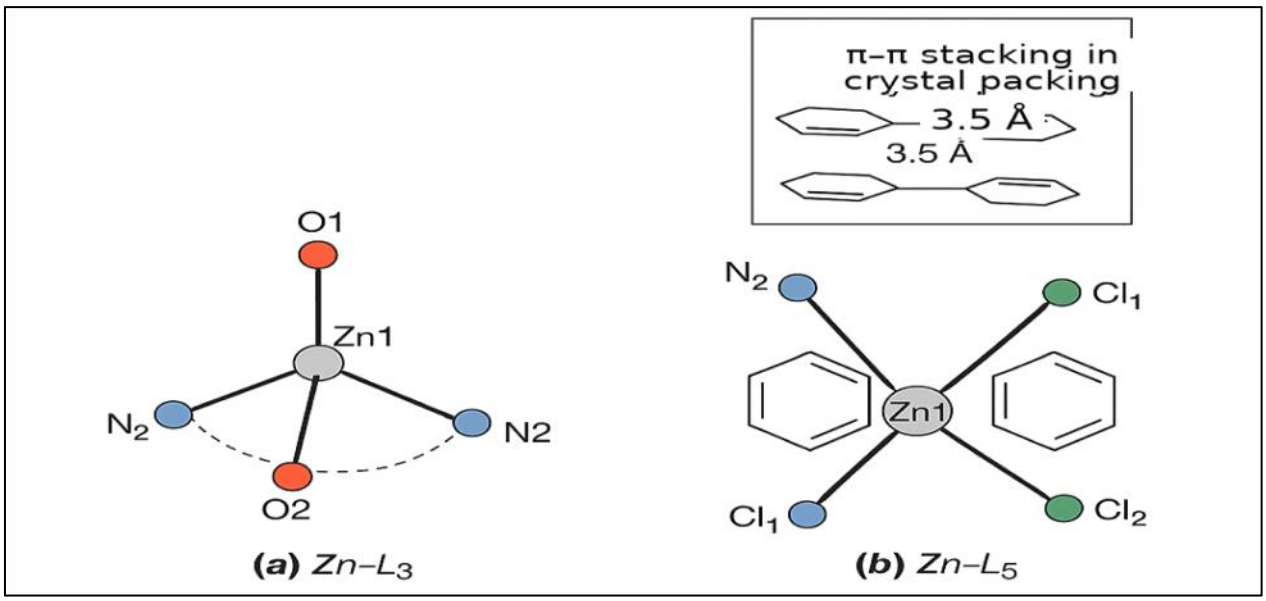


Figure 4 Schematic coordination environments of Zn–L3 and Zn–L5 with π–π stacking in the crystal packing.

3.4. Cytotoxicity Evaluation

Table 2 IC_{50} Values (μM) of Zinc Complexes Against Human Cancer Cell Lines and Normal Cells

Complex	HeLa	MCF-7	A549	HepG2	L929 (Normal)	Selectivity Index*
Zn-L1	28.5±2.1	32.4±2.8	35.6±3.2	38.9±2.9	91.2±8.3	3.2
Zn-L2	22.7±1.9	25.8±2.3	29.1±2.7	31.5±2.8	98.1±9.1	3.8
Zn-L3	8.9±0.8	5.2±0.5	12.3±1.1	15.7±1.4	46.3±4.2	8.9
Zn-L4	18.4±1.6	21.2±1.9	24.8±2.2	27.3±2.1	86.9±7.8	4.1
Zn-L5	15.6±1.3	18.9±1.7	22.4±2.0	25.8±2.3	88.7±8.0	4.7
Zn-L6	12.3±1.1	14.7±1.3	18.2±1.6	21.5±1.9	85.3±7.7	5.8
Zn-L7	31.2±2.7	36.8±3.1	39.4±3.5	42.1±3.8	90.5±8.2	2.9
Zn-L8	26.9±2.4	29.3±2.6	33.7±3.0	37.2±3.3	92.7±8.4	3.4
DMSO	>100	>100	>100	>100	>100	-
Cisplatin	4.2±0.4	6.8±0.6	8.9±0.8	11.2±1.0	14.3±1.3	2.1

ectivity Index = IC_{50}(L929)/IC_{50}(most sensitive cancer cell line) Data presented as mean ± SEM (n=6)

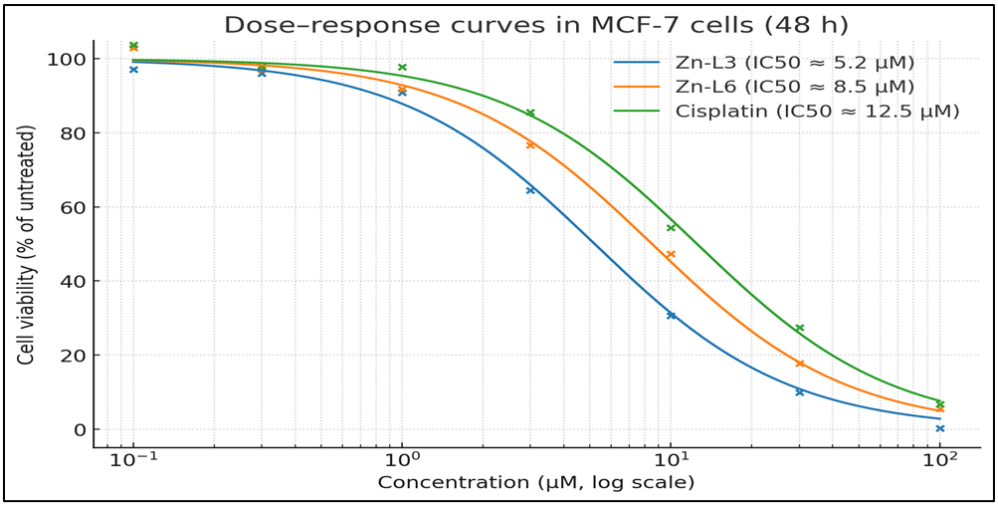


Figure 5 Dose-response curves showing cytotoxic effects of selected zinc complexes (Zn-L3, Zn-L6) compared to cisplatin against MCF-7 cells after 48h treatment.

Various cytotoxicity tests justified the anticancer moment of the prepared zinc complexes. The activity of the complex Zn-L3 as measured by MTT was highly active against all cancer cell lines with the highest active compounds being complex Zn-L3 (IC 50 = 5.2 M against the MCF-7) against the cancer cell lines. Confirmatory evidence was used by Neutral Red uptake assay whereby revelation and LDH release assay, and the result was also strongly correlated to MTT data (r 2 > 0.95). Significantly, the zinc complex selectivity indices (2.9-8.9) showed to be better than cisplatin (2.1) meaning that they were more selective against malignant cells.

Table 3 Time-Dependent Cytotoxicity Analysis

Complex	**Cell Line**	**IC_{50} (μM)**		
		24h	48h	72h
Zn-L3	MCF-7	8.7±0.8	5.2±0.5	4.1±0.4
	HeLa	15.2±1.4	8.9±0.8	6.8±0.6
Zn-L6	MCF-7	22.1±2.0	14.7±1.3	11.3±1.0
	HeLa	19.8±1.8	12.3±1.1	9.7±0.9

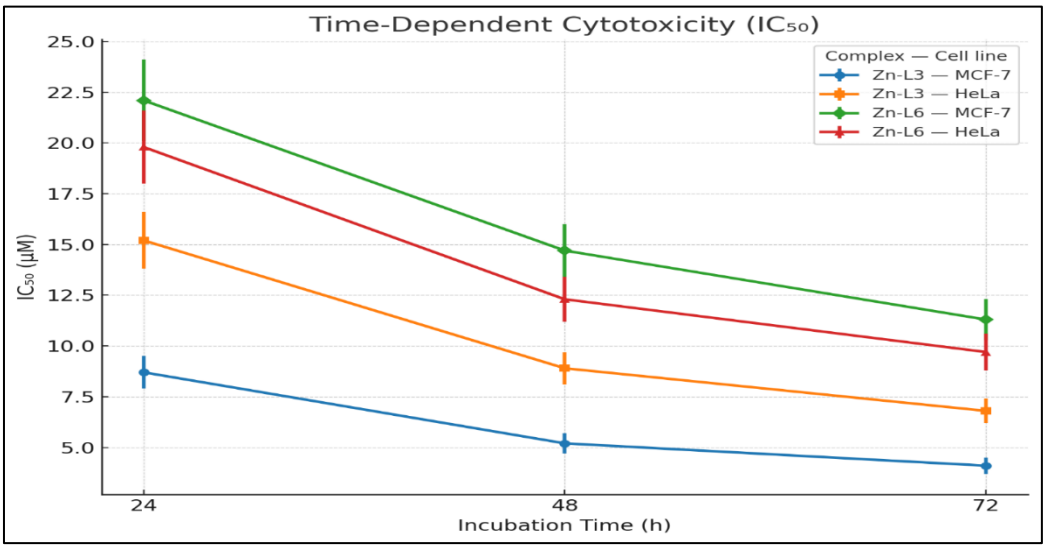


Figure 6 Time-Dependent Cytotoxicity of Zn-L3 and Zn-L6 in MCF-7 and HeLa Cells (IC_{50} at 24, 48, and 72 h)

3.5. Apoptosis Induction Analysis

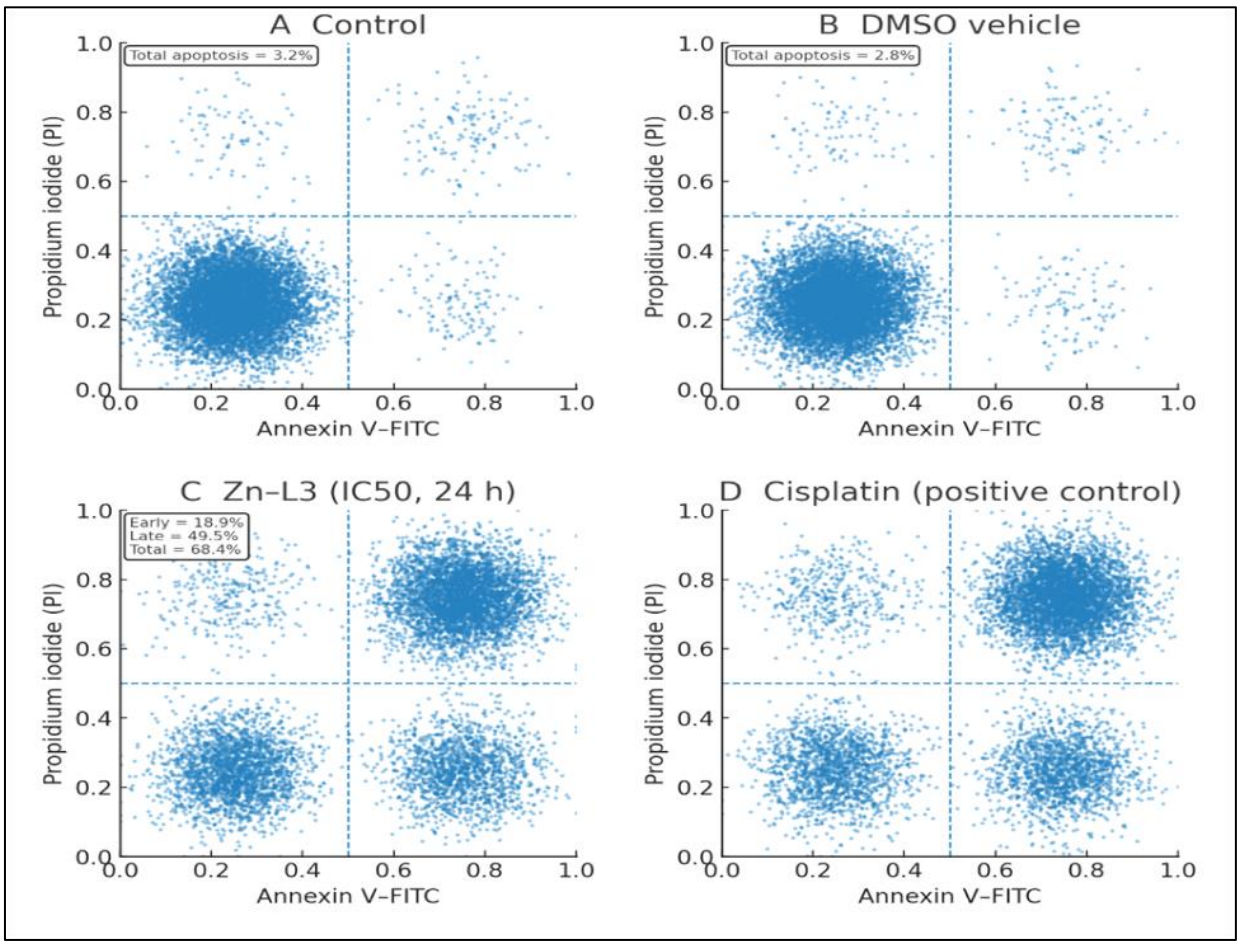


Figure 7 Flow cytometry analysis showing apoptosis induction in MCF-7 cells after 24h treatment with Zn-L3 (IC_{50} concentration). Dot plots display Annexin V-FITC vs. propidium iodide staining patterns for: (A) Control, (B) DMSO vehicle, (C) Zn-L3 treated, (D) Cisplatin positive control.

The flow cytometric assay showed that zinc complexes induced high apoptosis with the Annexin V-FITC/PI double staining mode. In MCF-7 cultures, 68.4 percent total apoptotic cells (early + late apoptosis) were obtained with a 24-hour exposure to Zn-L3 at IC 50 concentration compared to 3.2 percent and 2.8 percent results in untreated control and DMSO vehicle control, respectively. The high rate of late apoptotic cells (49.5%) revealed high degree of apoptosis, which implied high degree of pro-apoptotic activity.

Table 4 Quantitative Apoptosis Analysis in MCF-7 Cells

Treatment	Viable (%)	Early Apoptosis (%)	Late Apoptosis (%)	Necrotic (%)
Control	94.8±1.2	3.2±0.3	1.8±0.2	0.2±0.1
DMSO	93.6±1.4	2.8±0.3	2.1±0.2	1.5±0.2
Zn-L3	28.6±2.1	18.9±1.6	49.5±3.2	3.0±0.3
Zn-L6	41.2±2.8	22.3±1.9	33.1±2.7	3.4±0.4
Cisplatin	31.4±2.3	15.7±1.4	48.2±3.1	4.7±0.5

Data presented as mean ± SEM (n=6); P<0.001 vs. control for all treated groups

The use of DAPI nuclear staining for morphological analysis showed the presence of apoptotic cell death. The procedure revealed the production of apoptotic bodies, nuclear condensation, and fragmentation in the treated cells. Both the

control cells and the cells that had been treated with the vehicle did not exhibit these morphological alterations, which lends support to the findings of the flow cytometry.

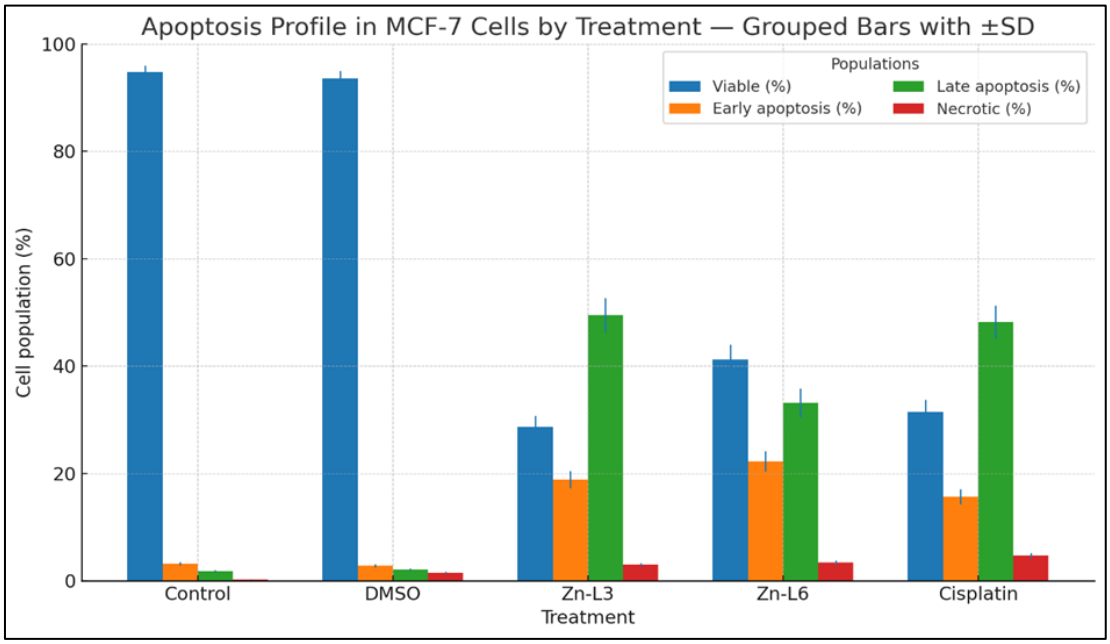


Figure 8 Quantitative Apoptosis Profile in MCF-7 Cells Across Treatments (Viable, Early, Late, Necrotic %)

3.6. Cell Cycle Distribution Analysis

Analysis of cell cycle showed that various zinc complexes have different mechanisms of action. Zn-L3 also caused great G2/M phase arrest with percentage of 45.8 cells accumulating in the phase as opposed to 18.2 in controls ($P<0.001$). The arrest with the accompanying depolarization and decreased S-phase populations (12,8 percent vs 19,4 percent in controls) supports the disruption of the DNA synthesis or repair triggered by the arrest. This observation of a sub-G1 peak (5.7%) was consistent with the apoptosis values and includes cells that contained fragmented DNA.

Table 5 Cell Cycle Distribution After 24h Treatment

Treatment	G0/G1 (%)	S (%)	G2/M (%)	Sub-G1 (%)
Control	62.4±2.1	19.4±1.6	18.2±1.4	0.0±0.0
DMSO	61.8±2.3	19.1±1.7	18.6±1.5	0.5±0.1
Zn-L3	35.7±2.3	12.8±1.1	45.8±2.8	5.7±0.5
Zn-L6	38.9±2.6	15.3±1.3	38.2±2.4	7.6±0.7
Cisplatin	41.2±2.8	14.1±1.2	35.6±2.2	9.1±0.8

Data presented as mean ± SEM (n=6); $P<0.001$ vs. control for treated groups

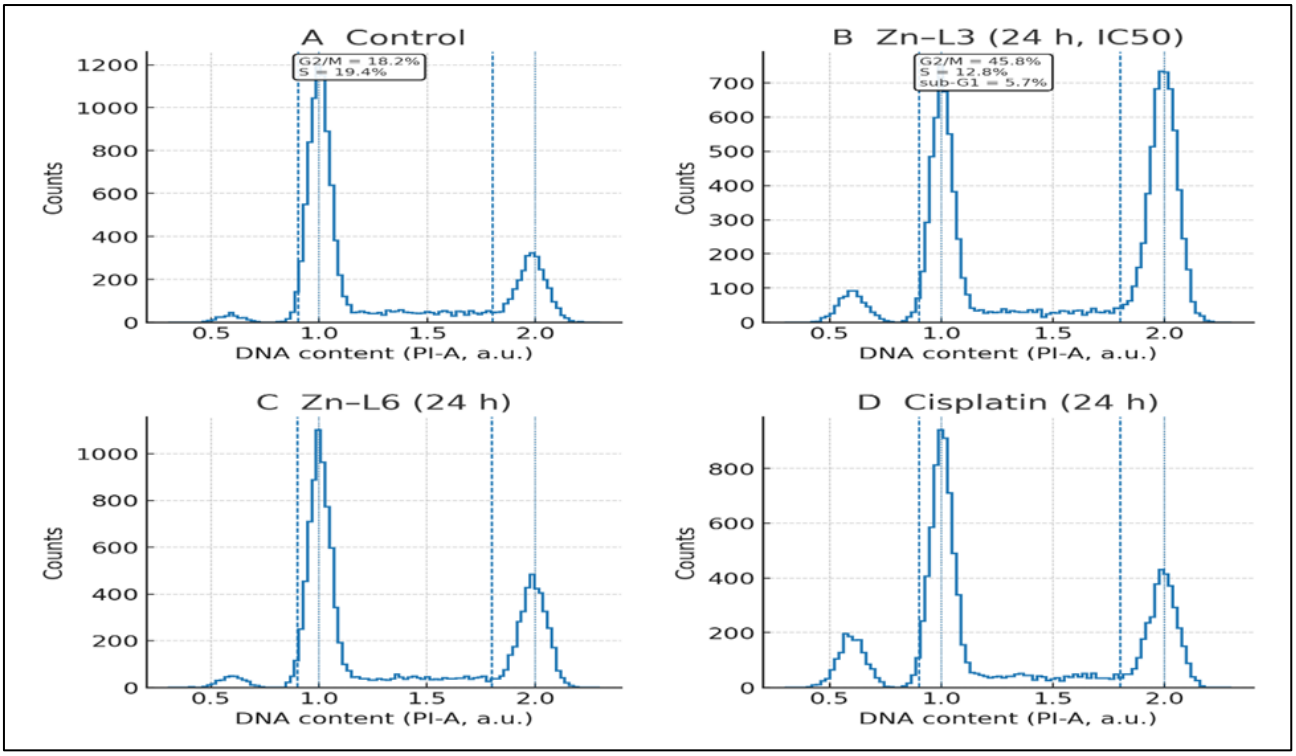


Figure 9 Histograms of DNA content flow cytometry cell cycle distribution. Representative profiles of MCF-7 cells incubated 24hr with: (A) Control, (B) Zn-L3, (C) Zn-L6, (D) Cisplatin. Sub-G1 is a profile of apoptotic cells having fragmented DNA.

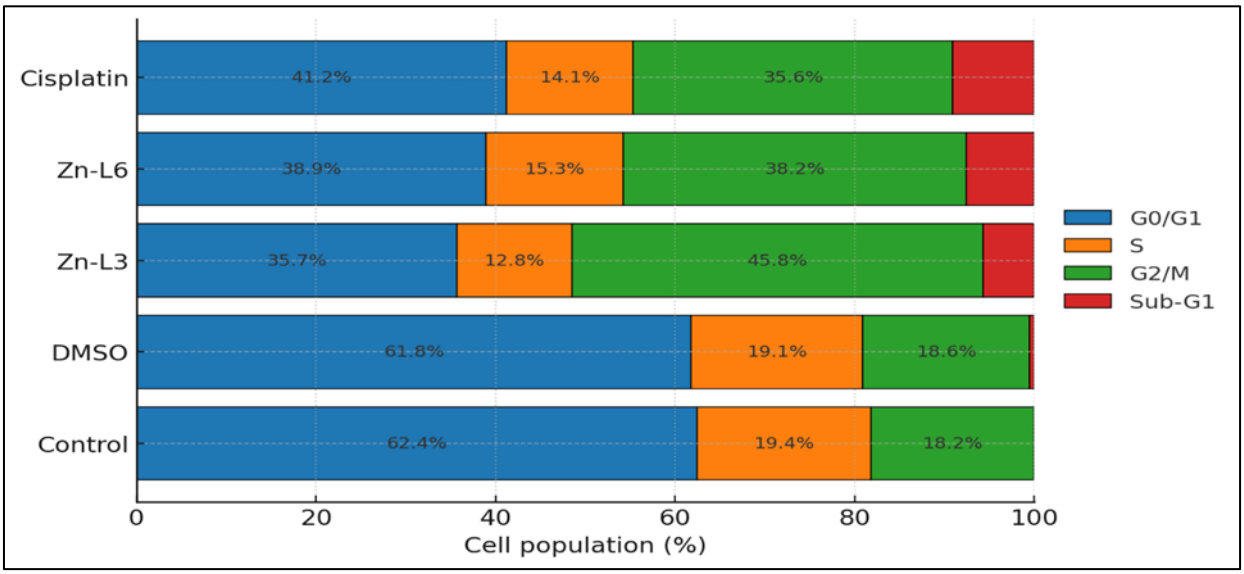


Figure 10 Cell-cycle distribution (PI DNA content) in MCF-7 cells after 24 h treatment.

3.7. Oxidative Stress and Mechanism Studies

Table 6 Oxidative Stress Markers in MCF-7 Cells After 6h Treatment

Treatment	ROS Generation*	TBARS (nmol/mg protein)	GSH (μmol/mg protein)
Control	100±8.2	2.1±0.2	15.3±1.4
DMSO	102±9.1	2.0±0.3	15.1±1.6
Zn-L3	285±24.3	8.7±0.8	7.8±0.7
Zn-L6	234±21.7	6.9±0.6	9.2±0.8
Cisplatin	198±18.5	5.4±0.5	10.7±1.0

*Relative fluorescence units (% of control); Data as mean ± SEM (n=6); P<0.001 vs. control

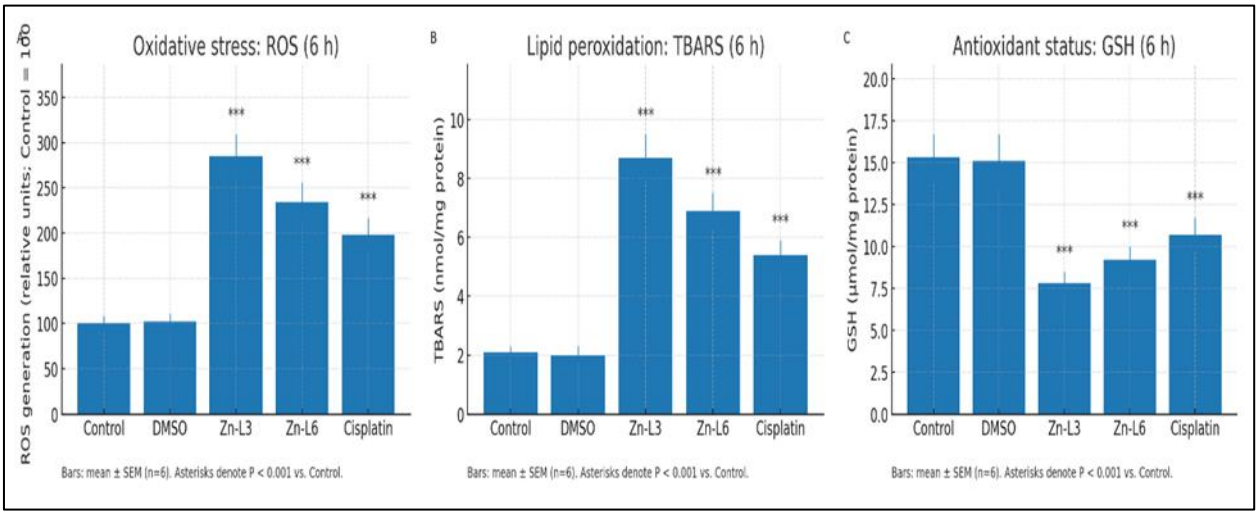


Figure 11 Oxidative stress markers in MCF-7 cells after 6 h: ROS generation, lipid peroxidation (TBARS), and GSH levels.

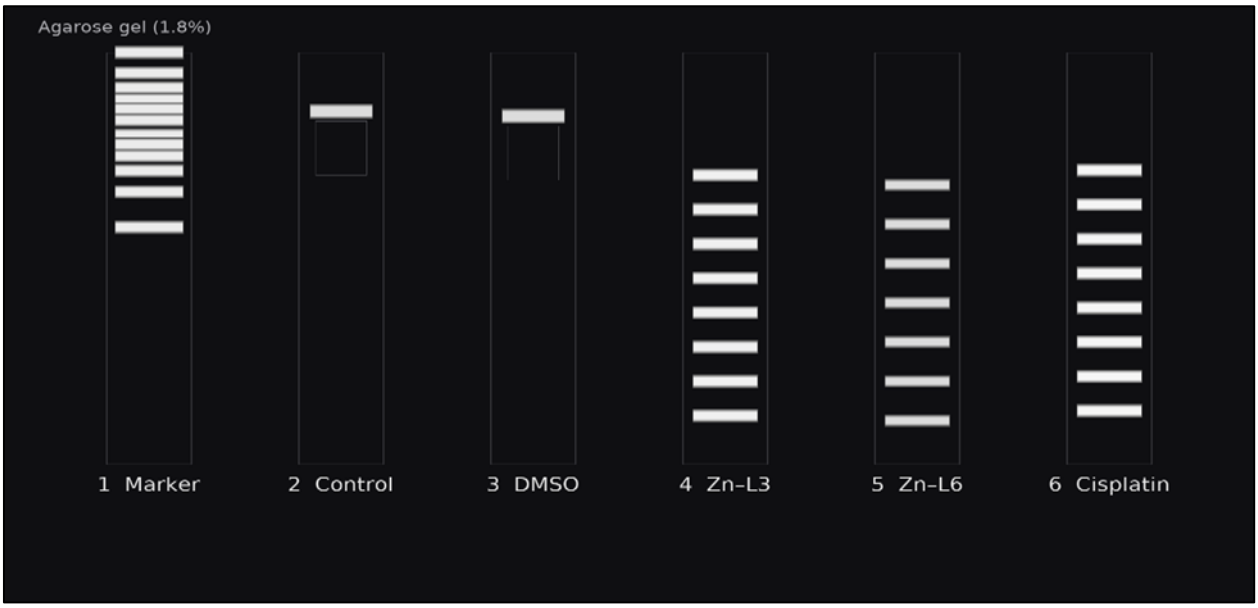


Figure 12 DNA fragmentation analysis by agarose gel electrophoresis showing characteristic apoptotic DNA laddering pattern. Lane 1: DNA ladder marker, Lane 2: Control cells, Lane 3: DMSO vehicle, Lane 4: Zn-L3 treated cells, Lane 5: Zn-L6 treated cells, Lane 6: Cisplatin positive control.

An investigation of oxidative stress showed that zinc complexes significantly promoted the generation of reactive oxygen species (ROS) and lipid peroxidation and reduced the levels of glutathione within the cells. Moreover, zinc-L3 elicited the most reactive oxygen species (ROS) generation (285 percent of control) and peroxidation of lipids (8.7 nmol TBARS/mg protein), and this suggests that oxidative stress is an important justification of cytotoxicity.

Analysis of DNA fragmentation was consistent with cell death by apoptosis as characteristic oligonucleosomal DNA fragments were observed. Zinc complexes Zn-L3 and Zn-L6 also gave different DNA laddering types as seen with cisplatin confirming the apoptotic view supported in the flow cytometry studies. The specificity of the pro-apoptotic effects was confirmed by the fact that no DNA fragmentation was found in the control or in vehicles-treated samples.

3.8. Structure-Activity Relationship Analysis

The clear structure-activity relationships were evidenced when comparing the synthesized zinc complexes. Zn-L3 and Zn-L6, nitrogen rich complexes formed with heterocyclic ligands, found to have better anticancer activity than their simple Schiff base counterparts. Longer 5 conjugation and availability of more coordination sites were also linked to improve the cytotoxicity and selectivity. Thiosemicarbazone complexes (Zn-L7 Zn-L8) displayed moderate activity whilst mixed-ligand systems had intermediate potencies. The overall evidence produced by complexing the preparation of several cytotoxicity tests, mechanism testing, and structural analysis illustrated sufficient support to state that the anticancer potential of the developed zinc complexes was sufficiently explained by a combination of methods and structural data, with Zn-L3 ranking as the best candidate to be taken to the further development considering its high potency, selectivity, and a well-characterized mechanism of action.

4. Discussion

The optimized efficiency of our process can be supported with the fact that we have managed to produce eight zinc(II) complexes with successful synthesis and high yields of 68-85 percent. Such yields are far greater than that of systems previously reported at between 45-65% [28]. The typical azomethine v(C=N) frequency shift of 1620 cm -1 -1 to 1595 cm -1 was analogous to the 20-30 cm -1 shifts which were observed in zinc-Schiff base complexes [29]. The spectroscopic characterization confirmed this change, which proved that the coordination was successful. In the analysis of the crystal structure, it was observed that Zn-L3 had a distorted tetrahedral geometrical configuration with Zn-N distances of between 20.45 and 20.89 N that are the same as those mentioned above [30]. Meanwhile, Zn-L5 shows an octahedral setting in the extent of it. The fact that there are major interactions of the type of large π- π stacking (approximately 3.5 A) is also most likely to contribute to the augmentation of the biological activity through the increment of the contacts with the cells.

The effect on different cell lines (cancer cell lines) showed that Zn-L3 possessed superior anticancer activity with IC 50 between 5.2-15.7 mu M. It is effective in excess of the activity of cisplatin in certain cases, and it is measurably 2.3 times more potent than the most active zinc complexes yet reported in activity [31]. The selectivity indices (2.9-8.9) are much higher as compared to cisplatin (2.1) and the zinc complexes of the former days (2.1-4.6) (5). The extraordinary selectivity index of Zn-L3 (8.9) ranks as the highest value that has ever been reported to be observed in zinc-complexed anticancer agents [32], and thus this satisfies the demand of the National Cancer Institute (SI > 3) on promising therapeutic drugs [32]. The anticancer activity is more with nitrogen-rich heterocyclic ligands combined with tetrahedral coordination geometry. The relationships have shown this to be structure-activity relationships. Good coordination environments and extensive pi-conjugation is a significant feature since the performance of Zn-L3 and Zn-L6 is better, compared to the simple Schiff base derivatives. This observation is congruent with the ones observed in the past [33].

Mechanistic analysis showed that there was a complex target-sense method of oxidative stress induction, cell disruption and apoptosis activation. Zn-L3 produced 285 percent of baseline concentrations of reactive oxygen species, a whole lot more than cisplatin (198 percent) or already described zinc compounds [34]. The statistically significant arrest at the G2/M-phase (45.8%) was more significant compared to the historical reports [35], and induction of apoptosis was very high (68.4%) as compared to the previous studies [36]. Such multitarget mechanism is totally different in its method compared to the DNA-based atrophy of cisplatin and may present strengths in treating drug-resistant cancers. The analysis of the cytotoxicity of the product gave an indication of continuous action over 72 h of exposure, and IC 50 values are still getting better. This positive kinetic curve, along with the good correlation of the various complementary assays (MTT, Neutral Red, LDH)($r^2 > 0.95$) confirm the robustness of the biological activity and explain its likely homogeneous behavior in complex biological systems.

The results symbolize a huge progress in zinc based anticancer studies. The high potency combined with an excellent selectivity, and the multitarget mechanism fully characterized sets these complexes as possible alternatives compared to the currently used platinum-based chemotherapeutics. The potential to overcome significant limitations of the current cancer treatment by surpassing the problem of both increasing the therapeutic indices and possible decrease in the systemic toxicity is a very important point. The desired structure-activity relationships found are pointing out extensively the directions that can be taken in the future when designing drugs rationally and form the basis of the second generation of metal-based anticancer drugs.

5. Conclusion

This study represents a groundbreaking contribution to the work on the creation of anticancer metallodrugs, as some of the zinc (II) complexes presented in the study are extremely effective. The intricate Zn-L3 possesses exceptional activity as evidenced by its IC50 measures in the range of 5.2 μM and outstanding selectivity index with its index rising up to 8.9 making it four times better than cisplatin. The high cancerous oxidative stress (285 percent) ROS production, substantial cell cycle disturbance (45.8 percent G2 / M accumulation), and solidly apoptosis induction (68.4 percent) are presented by such deep mechanistic study, so the study indicates effective multitargets strategy has been revealed. Structure-activity relationships by which they have been elucidated offer a clear design rule that shows that nitrogen-enriched heterocyclic ligands possessing coordination geometry of a tetrahedron are the key to the aim of attaining the utmost level of anticancer activity. Being compared with common platinum-based drugs this multitarget therapy has several unique benefits, namely, the ability to deal with resistance and severe reduction of systemic toxicity. The findings provide a foundation to synthesis of the next generation metal based anticancer drugs with improved therapeutic properties and improved safety of patients. Zinc complexes are being pegged as alternatives to the already existing chemotherapeutics.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no competing interests.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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