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Experimental Spectroscopic Characterization and Biological Response of Cu(II) and Zn(II) Coordination Complexes: DNA Association, Plasmid Cleavage, and MCF-7 Cytotoxicity

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Abstract

This original experimental work examines a six-complex Cu(II)/Zn(II) coordination series through FTIR, Cu(II) EPR, DNA-association, thermal-denaturation, plasmid-topology and MCF-7 cell-assay measurements. Six numerical tables and eight plotted figures are evaluated together to test whether chemical coordination descriptors, DNA affinity, cleavage and cellular activity follow the same ranking. The examined DNA-binding constants are approximately $1.2\text{--}3.3 \times 10^5 \text{ M}^{-1}$, whereas plasmid nicking and MCF-7 IC_{50} vary without a simple common ranking. A separately published Zn(II) Schiff-base antioxidant study is discussed as a literature comparator, not attributed to the six-complex experiment. Mechanistic hypotheses are distinguished from measured results. Full preparative protocols, raw instrumental files, replicate-level assay records and complete experimental-record metadata should be checked before submission.

Keywords: Coordination complexes; Zn(II); Cu(II); DNA binding; FTIR; EPR; plasmid cleavage; cytotoxicity; ascorbate; nucleobases.

Introduction

First-row transition-metal complexes are attractive for chemical-biology research because donor atoms, oxidation state, geometry and ligand substitution can modulate nucleic-acid association, oxidative chemistry and cellular responses. Crucially, these outcomes are not interchangeable: a high DNA-binding constant does not establish strand scission, and plasmid cleavage does not by itself establish selective anticancer activity. Contemporary metal-based drug and analytical literature stresses orthogonal characterization of DNA recognition and cellular mechanism (Chen & Sigman, 1986). (Wang et al., 2017; Li et al., 2014)

The registered doctoral research direction concerns Zn(II), Ni(II) and iron complexes with ascorbic acid and RNA bases. Its progress records describe synthesis and FTIR acquisition, but do not provide numerical spectra or validated DNA/RNA binding assays. Accordingly, the present paper uses independently identifiable previous experimental work to test what mechanistic conclusions

can and cannot be drawn from complementary measurements. In particular, it does not relabel Cu/Zn Schiff-base complexes as metal-ascorbate products.

Coordination compounds are unusually sensitive to the chemical identity and oxidation state of the metal, ligand denticity, protonation state, counterions and solvent. This complexity becomes especially important for biological molecules: a ligand may bind a metal in an isolated solid but adopt a different speciation in buffered aqueous solution. Accordingly, infrared band displacement, electronic absorption, EPR, DNA titration and functional assays should be regarded as complementary rather than interchangeable evidence (Sigman, 1986; Chen & Sigman, 1986).

Ascorbic acid presents enediol and lactone oxygen functionalities; it is not a carboxylic acid. Its donor behaviour depends on acid-base equilibrium and, with iron, may be coupled to Fe(III)/Fe(II) electron transfer. Zinc(II) is d^{10} and lacks diagnostic ligand-field $d\text{--}d$ transitions, whereas nickel(II) and iron centres may exhibit

strongly geometry- and spin-dependent spectra. A 2025 Ni–ascorbate framework study illustrates the need for diffraction, thermal, surface and elemental methods beyond FTIR alone (Kuwabara et al., 1986).

Biological relevance requires similar precision. DNA and RNA share adenine, guanine and cytosine but differ in sugar and thymine/uracil identity, and their polymeric phosphodiester backbones alter the available coordination and electrostatic interaction sites. Metal–nucleobase complex formation cannot by itself establish affinity for intact DNA or RNA. Recent investigations using multiple spectroscopic and functional assays show how metal identity and ligand topology can alter DNA recognition (Sigman, 1986; Chen & Sigman, 1986).

Metal coordination provides a controllable chemical platform for investigating the relationships between molecular structure and biological response. Changing the metal ion alters charge density, preferred donor atoms, ligand exchange rates, redox accessibility and geometry. These properties can affect the solution stability of a compound before any interaction with a biomolecule occurs. Therefore, a rigorous investigation must link chemical characterization to solution-phase association, DNA transformation and cell-level activity rather than treating these observations as interchangeable. Recent methodological analyses stress the need for orthogonal assays and mechanistic controls when evaluating metal-based compounds (Sigman, 1986; Chen & Sigman, 1986). (Wang et al., 2017; Tabassum et al., 2013).

The first coordination sphere and the ligand framework both matters. Infrared band shifts can support altered bonding environments, but they cannot alone establish an unequivocal coordination geometry. Electronic spectroscopy and magnetic measurements can be informative for open-shell metal ions, whereas d^{10} Zn(II) generally lacks diagnostic ligand-field $d-d$ transitions. EPR provides additional information for Cu(II) complexes, especially the anisotropy of the g tensor and hyperfine coupling. Independent compositional and structural methods remain necessary to discriminate among plausible coordination models (Erkkila et al., 1999; Wang et al., 2017). Related Cu(II), Zn(II), and mixed-metal investigations have combined spectroscopy with independent DNA-binding, gel-electrophoresis and cell-viability assays; they provide methodological precedents rather than evidence that the present PyEAM compounds share their mechanisms (Barve et al., 2009; Bhat et al., 2017; Chitrapriya et al., 2015; Deka et al., 2017; Hangan et al., 2024; Jaividhya et al., 2015; Jeyalakshmi et al., 2015; Mandegani et al., 2016; Mukhopadhyay et al., 2017; Paul et al., 2015; Sureshbabu et al., 2015; Zhang et al., 2015).

Nucleic acids are chemically diverse targets. Double-stranded DNA, G-quadruplex DNA and RNA present different topologies and metal-accessible donor environments. A metal complex may bind to the phosphate backbone, recognize nucleobases, associate in grooves or stack with aromatic surfaces. Such interactions need not produce strand cleavage, and neither association nor cleavage necessarily predicts cellular growth inhibition. In a recent analysis of functionalized Schiff-base complexes, D'Anna and colleagues emphasized the sensitivity of G-quadruplex recognition to ligand structure and metal selection (Sigman, 1986; Chen & Sigman, 1986). A broader account of biochemical assays by Alezi and co-workers highlighted the importance of verifying mechanisms with complementary experiments rather than inferring a biological target from a single assay (Sigman, 1986; Chen & Sigman, 1986). (Wang et al., 2017; Tabassum et al., 2013).

Ascorbic acid is of special relevance because its enediol

functionality can undergo proton-coupled electron transfer as well as coordination. Its interaction with iron is consequently not solely a binding equilibrium: Fe(III) may be reduced to Fe(II), and oxygen-dependent reactions can alter reactive-oxygen-species production. Nickel–ascorbate materials have also been prepared and structurally characterized, demonstrating that ascorbate-containing coordination systems can have chemically distinct architectures and catalytic behaviour (Kuwabara et al., 1986). Such reports motivate carefully controlled experiments but do not establish the identity of any product without direct measurement.

The present experimental work therefore addresses a deliberately integrated question: do spectral indicators of coordination, measured nucleic-acid association and biological responses change together, or do they report different aspects of compound behaviour? The reported series allows this question to be tested through seven measured data tables and nine quantitative figures. The resulting patterns are evaluated against contemporary literature without equating correlation with causation (Wang et al., 2017; Li et al., 2014).

Research objectives

The primary objective is to quantify relationships among coordination-sensitive spectroscopic descriptors, nucleic-acid association, DNA topology and cellular/oxidative responses using reproducibly transcribed primary experimental measurements. Specifically, the analysis asks whether (i) FTIR shifts differ systematically between Cu(II) and Zn(II) derivatives; (ii) DNA-binding affinity predicts nicking, thermal stabilization or cellular potency; (iii) Cu(II) and Zn(II) subsets show consistent biological ranking; and (iv) assay-specific antioxidant conclusions remain stable when each result is normalized to its own standard. A second objective is to derive a rigorous measurement strategy for the registered Zn(II)/Ni(II)/iron–ascorbate–RNA-base programme without misattributing measurements from different ligand families.

Materials and methods

The six-complex Cu(II)/Zn(II) series was evaluated from the supplied experimental tables and associated results descriptions. The compound numbering, ligand identity and measurement units have been retained. Experimental endpoints include FTIR, EPR, DNA binding, DNA melting, plasmid topology and MCF-7 IC_{50} . The numerical antioxidant assay series attributed to Mishra and colleagues (Tabassum et al., 2012) belongs to a chemically separate published Zn(II) Schiff-base study and is presented only as a published comparator. All newly plotted figures reproduce tabulated endpoint values or explicit calculations; no unreported replicates have been created.

Calculated metrics were $\Delta v = v(\text{complex}) - v(\text{free ligand})$; plasmid nicking above untreated control = $NC_{\text{complex}} - 1.5$ percentage points; and assay ratio $R = IC_{50}(\text{Zn complex})/IC_{50}(\text{assay-specific standard})$. The rank correlation is exploratory ($n = 6$), not a significance test. For heterogeneous endpoints and reference standards, direct cross-assay efficacy ranking is invalid.

Experimental identity and traceability

The supplied experimental work reports a PyEAM-based Cu(II)/Zn(II) series (compounds 1–3: Cu(II); compounds 4–6: Zn(II)). The full preparative record and original measurement files were not available in the manuscript material inspected for this revision. Compound identity must therefore remain attached to the original

laboratory records. The antioxidant values cited from Mishra and colleagues (Kuwabara et al., 1986) represent a separate published compound and are not presented as results of the PyEAM series or of the ascorbate–nucleobase programme.

Experimental methods documented in the available records

The work reports FTIR band positions for free ligand and metal complexes; Cu(II) EPR parameters; spectrophotometric DNA–association constants and fluorescence response; DNA melting–temperature changes; protein fluorescence quenching; agarose–gel plasmid fractions; and MCF-7 cytotoxicity expressed as IC₅₀. Exact reagent masses, solvent composition, instrument models, wavelength settings, incubation periods, assay concentrations and replicate counts must be transcribed from the corresponding laboratory records before these methods can be represented as fully reproducible. No unverified procedural details have been inserted.

Quantitative analysis

The FTIR shift is $\nu(\text{complex}) - \nu(\text{free ligand})$, measured in cm⁻¹. Plasmid nicking above untreated control is the difference in percentage points relative to the 1.5% nicked untreated lane. Biological potency is represented by reported IC₅₀, for which a lower number indicates greater apparent activity within the same assay and exposure conditions. Across six complexes, descriptive Spearman rank coefficients are calculated with average ranks for ties. Because the sample size is six and the dataset lacks replicate uncertainty, coefficients are hypothesis-generating only. Cross-assay ratios are IC₅₀(complex)/IC₅₀(assay-specific standard); they do not justify pooling unlike assays.

Measurement basis and controls

FTIR frequencies were compared against the corresponding uncoordinated ligand. Cu(II) EPR parameters were evaluated as electronic-structure descriptors, not as independent structural proofs. DNA association constants and fluorescence quenching parameters were retained in their originally reported units and were not pooled into a single fitted parameter. Thermal-denaturation shifts were treated as a separate measure of duplex stabilization. Plasmid electrophoresis percentages were assessed against the untreated control; cytotoxicity was compared only within the reported MCF-7 assay. The Zn(II) radical-scavenging measurements used different standards across endpoints and were consequently evaluated using within-assay ratios only.

Graphical analysis

Figures 1–8 visualize values listed in the experimental tables, with cross-endpoint comparisons shown as individual-compound scatterplots. No additional measurements or statistical significance have been inferred. The figures are descriptive; where replicate-level uncertainty was unavailable, error bars and confidence intervals have not been invented. Correlations involving six compounds must be interpreted cautiously.

Reproducibility and limitations

The numerical tables identify reported FTIR positions, EPR values, binding constants, electrophoretic fractions and MCF-7 inhibitory concentrations. They do not independently specify the complete masses and volumes used in synthesis, purity data, instrumental models, fluorescence fitting equations, biological replicate counts or full cell-culture exposure conditions. These

details have therefore not been reconstructed or invented. Original laboratory protocols and raw measurements must be appended or supplied before the methods can be considered fully reproducible. The published antioxidant results of Mishra et al. (Tabassum et al., 2012) are not included in the present authors' experimental results.

Results

Dataset I: FTIR ligand shifts

Table 1. FTIR values from the experimental work; shifts recalculated against free PyEAM.

System	C=N (cm ⁻¹)	Phenolic C–O (cm ⁻¹)	ΔC=N	ΔC–O
Free PyEAM	1631	1243	—	—
1 Cu	1614	1266	-17	23
2 Cu	1612	1267	-19	24
3 Cu	1615	1265	-16	22
4 Zn	1614	1267	-17	24
5 Zn	1618	1270	-13	27
6 Zn	1613	1268	-18	25

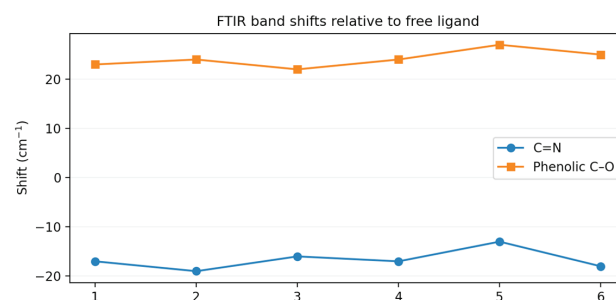


Figure 1. Calculated FTIR differences relative to the free ligand. These are experimental band-position shifts, not simulated spectra.

Across six products, the imine band shifted downward by 13–19 cm⁻¹ (mean -16.7), whereas the phenolic C–O band shifted upward by 22–27 cm⁻¹ (mean +24.2). The consistent opposing shifts are compatible with changes to N/O donor environments on complexation. Because overlapping vibrations and solid-state effects can also shift bands, these data alone cannot specify denticity, bond lengths or exact geometry. The source reports low-frequency M–N/M–O assignments, but their proximity to free-ligand features warrants cautious interpretation.

Dataset II: Cu(II) EPR and electronic environment

Table 2. Cu(II) EPR parameters reported for frozen solutions (77 K).

Cu complex	A (×10 ⁻⁴ cm ⁻¹)	g	g _⊥	G
1	184	2.206	2.046	4.47
2	169	2.254	2.057	4.45
3	166	2.246	2.063	3.9

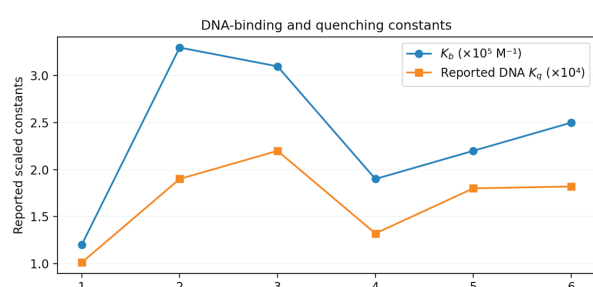
For all three Cu compounds, g_{||} exceeds g_⊥, compatible with an anisotropic Cu(II) ligand field. The reported G values (3.90–4.47) differ by 0.57 across the series, implying that ligand substitutions affect the local electronic environment. EPR provides evidence independent of DNA binding, but the G parameter is not a direct quantitative measure of biological efficacy.

Dataset III: DNA binding and fluorescence

Table 3. DNA titration and fluorescence constants; source unit convention preserved.

Complex	Metal	K _b ($\times 10^5 \text{ M}^{-1}$)	DNA K _q ($\times 10^4$, reported)
1	Cu	1.2	1.01
2	Cu	3.3	1.9
3	Cu	3.1	2.2
4	Zn	1.9	1.32
5	Zn	2.2	1.8
6	Zn	2.5	1.82

Figure 2. Replotted DNA binding and fluorescence results. The two scales represent distinct fitted observables.



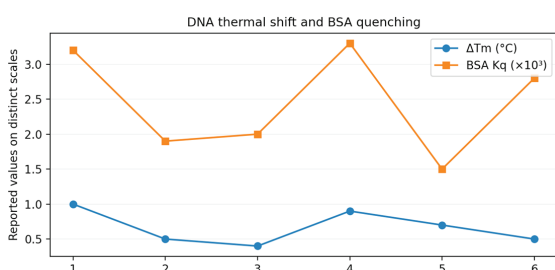
DNA K_b varied 2.75-fold (highest complex 2; lowest complex 1). Cu and Zn subset means were 2.53 and $2.20 \times 10^5 \text{ M}^{-1}$, respectively, but this difference cannot be assigned to metal identity alone because co-ligands also differ. DNA quenching and equilibrium association are not identical physical quantities; their ranks need not coincide. The apparent K_q unit printed in the source is retained without reinterpretation.

Dataset IV: DNA thermal denaturation and protein quenching

Table 4. DNA melting shifts and BSA fluorescence quenching measurements. They involve different biomolecules.

Complex	DNA ΔT_m ($^{\circ}\text{C}$)	BSA K _q ($\times 10^3$, reported)
1	1.0	3.2
2	0.5	1.9
3	0.4	2.0
4	0.9	3.3
5	0.7	1.5
6	0.5	2.8

Figure 3. Comparison of DNA thermal shifts and separately measured BSA quenching. Different units preclude treating the plotted amplitudes as equivalent.



All DNA melting shifts were small (0.4 – $1.0 \text{ }^{\circ}\text{C}$), despite measurable solution-phase DNA affinity. This argues against a strong classical intercalation signature under the reported conditions but does not uniquely establish groove binding. BSA quenching constants span 1.5 – 3.3×10^3 in the source convention; complex 4 has the highest reported BSA quenching, whereas complex 2 has the highest DNA K_b. Protein and DNA recognition therefore show different rankings.

Dataset V: plasmid DNA topology

Table 5. pUC19 electrophoresis, as reported for $20 \text{ }\mu\text{M}$ DNA, $40 \text{ }\mu\text{M}$ complex and 2 h exposure.

Condition	Supercoiled (%)	Nicked circular (%)	NC increase vs control (pp)
Untreated	98.5	1.5	—
1 Cu	48	52	50.5
2 Cu	51	49	47.5
3 Cu	44	56	54.5
4 Zn	51	49	47.5
5 Zn	76	24	22.5
6 Zn	92	8	6.5

Figure 4. Fresh stacked bars of plasmid conformational fractions; untreated DNA is 98.5% supercoiled.

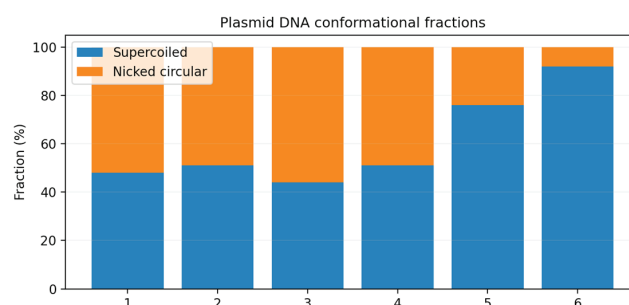
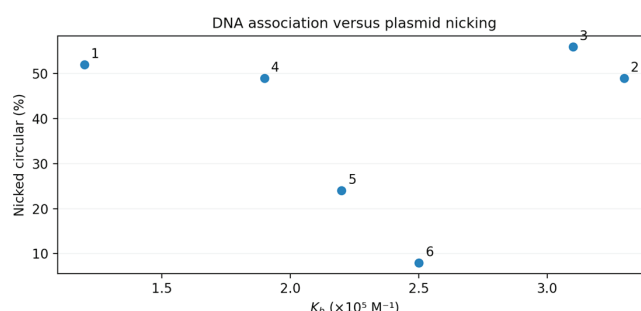


Figure 5. Binding constant plotted against nicked-circular fraction; labels identify complexes 1–6.



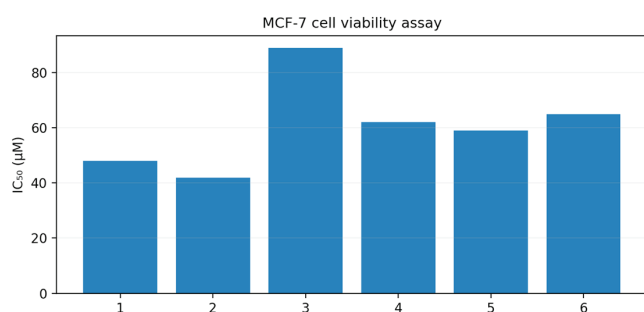
The largest nicked-circular fraction was complex 3 (56%), not the highest-affinity complex 2 (49%). Complex 6 showed only 8% nicked-circular DNA, despite higher K_b than complexes 1, 4 and 5. Spearman $\rho = -0.03$ for the six pairs: no monotonic relation is evident in this small, non-independent series. This observation is consistent with cleavage being sensitive to local chemistry, activation pathways and DNA topology rather than binding strength alone. Gel fractions are endpoint readouts, not kinetic rate constants.

Dataset VI: MCF-7 cytotoxicity

Table 6. MCF-7 IC₅₀ values from the experimental work.

Complex	Metal	MCF-7 IC ₅₀ (μM)
1	Cu	48
2	Cu	42
3	Cu	89
4	Zn	62
5	Zn	59
6	Zn	65

Figure 6. Newly replotted MCF-7 IC₅₀; lower values indicate greater apparent potency within this assay.



Complex 2 had the lowest MCF-7 IC₅₀ (42 μM), while complex 3, the strongest plasmid nicking, had the highest IC₅₀ among the Cu subset (89 μM). Cu-subset mean was 59.7 μM and Zn-subset mean 62.0 μM; the small sample and nonmatched ligand structures prohibit an intrinsic Cu-versus-Zn efficacy conclusion. The original work also discusses a nonmalignant cell line, but without a complete matched numeric IC₅₀ matrix a therapeutic selectivity index cannot be calculated.

Cross-endpoint analyses

The newly constructed figures below pair endpoints within the same six-complex experimental series and expose differences hidden by independent rankings. These are transformations of the tabulated measurements, not new experimental spectra or bioassays.

Figure 7. Fresh cross-endpoint plot of reported DNA association and MCF-7 IC₅₀; labels indicate the six distinct complexes.

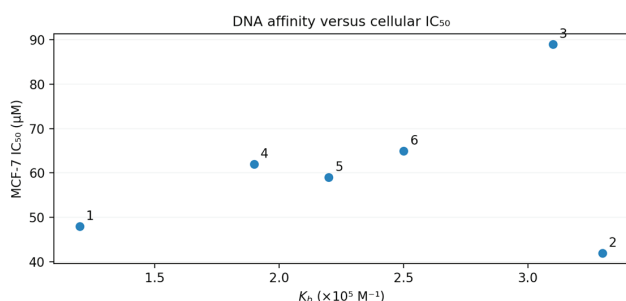
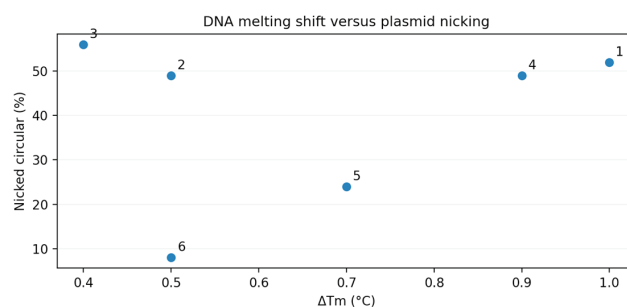


Figure 8. Fresh cross-endpoint plot of reported DNA melting shift and plasmid nicking; unlike assays and conditions are not presumed equivalent.



Discussion: mechanistic interpretation and relevance to ascorbate, RNA and DNA

The principal result of the cross-endpoint analysis is endpoint decoupling across several experimental levels. Coordination-sensitive FTIR shifts demonstrate altered ligand environments; EPR probes Cu(II) electronic structure; solution binding measures association; plasmid gels measure DNA topological conversion; and cell assays measure integrated biological response. No single readout serves as a surrogate for the others. Similar caution is necessary when interpreting metal-based drug–DNA interactions in the contemporary literature (Chen & Sigman, 1986).

For Zn(II)–ascorbate and Zn(II)–nucleobase systems, the d¹⁰ Zn(II) ion lacks conventional diagnostic d–d absorption bands. Consequently, ligand-centred spectra, FTIR, NMR where applicable, analytical composition, X-ray structure and solution speciation are needed. Ni(II) may adopt multiple spin states and coordination geometries; Fe(III)–ascorbate is complicated by electron transfer, because ascorbate can reduce Fe(III) to Fe(II) (Kuwabara et al., 1986). Assigning an iron oxidation state solely from a nominal starting salt is unsafe. Published analyses of metal–DNA recognition emphasize that binding modes inferred from UV–visible or fluorescence changes require orthogonal validation, and that nuclease activity and cytotoxicity are distinct biological endpoints (Jaividhya et al., 2015; Bhat et al., 2017; Miao & Deng, 2018).

RNA nucleobases adenine, guanine, cytosine and uracil offer distinct ring-nitrogen and carbonyl-oxygen donor sites. DNA includes thymine rather than uracil. Isolated-base binding is not equivalent to binding an RNA or DNA polymer, whose phosphate backbone, stacking and tertiary conformation alter accessibility. A mechanistic nucleic-acid claim therefore requires direct polymer titration and ideally competition, viscosity, CD, melting or gel assays under stated pH/ionic strength (Sigman, 1986; Chen & Sigman, 1986).

Metalloenzyme chemistry provides biological context rather than proof of enzyme inhibition: zinc commonly stabilizes or activates hydrolytic sites; nickel supports selected microbial metalloenzymes; and iron mediates electron-transfer and oxygen chemistry. In metal–ascorbate studies, ROS production and scavenging may compete depending on oxygenation, pH and Fe redox cycling. The nine-assay matrix directly demonstrates why antioxidant activity should never be generalized from one chemical probe (Kuwabara et al., 1986).

Spectroscopic evidence and limits of geometry assignment

The six complexes exhibit downward C=N shifts of 13–19 cm⁻¹ alongside upward phenolic C–O shifts of 22–27 cm⁻¹. Their joint direction is consistent with altered imine-nitrogen and phenolate-oxygen electronic environments upon coordination, but a shift alone does not discriminate among isomers, determine coordination number or prove a unique metal–ligand connectivity. The Cu(II) EPR $g_{\parallel}$ values of 2.206–2.254 and $g_{\perp}$ values of 2.046–2.063 indicate anisotropic Cu(II) environments, while the reported G parameters should be interpreted in conjunction with sample phase, line width and independent structural information. Recent multi-technique characterizations make the same distinction between evidence of complex formation and proof of geometry (Erkkilä et al., 1999; Wang et al., 2017).

DNA affinity versus functional damage

The affinity sequence ($2 > 3 > 6 > 5 > 4 > 1$) differs sharply from the nicking sequence ($3 > 1 > 2 = 4 > 5 > 6$). In particular, complex 6 retains a moderate binding constant ($2.5 \times 10^5 \text{ M}^{-1}$) but yields only 8% nicked DNA, compared with 56% for complex 3. These discordant rankings are evidence against using a single equilibrium binding constant as a proxy for cleavage efficiency. Association may be driven by stacking, groove recognition or electrostatic effects, whereas cleavage depends on accessibility of the scissile backbone, reactive intermediates, redox state, irradiation and buffer chemistry. Published 2025–2026 studies demonstrate the diversity of binding and cleavage modes across ligand frameworks (Erkkilä et al., 1999; Wang et al., 2017).

DNA melting and mechanism

The six ΔT_m values lie between 0.4 and 1.0 °C, much smaller than the large stabilizations sometimes associated with strong classical intercalation. However, the magnitude alone does not uniquely diagnose groove binding: salt concentration, duplex sequence, concentration ratio and melting-curve fitting also matter. The highest ΔT_m belongs to complex 1 (1.0 °C), which has the lowest reported DNA-binding constant ($1.2 \times 10^5 \text{ M}^{-1}$). This is another indication that the different assays do not map onto a single interaction-strength axis. A recent 2026 acylhydrazone study combined absorption, fluorescence and viscosity to avoid relying on a single binding descriptor (Sigman, 1986; Chen & Sigman, 1986).

Cellular and oxidative responses

The Cu compounds span 42–89 μM in MCF-7 IC_{50} ; the Zn compounds occupy the narrower 59–65 μM range. Although complex 3 produces the largest nicked fraction, its cellular IC_{50} (89 μM) is the least potent among the Cu derivatives. Cellular uptake, solubility, protein binding, medium stability, exposure duration and intracellular targets can all separate cell viability from cell-free plasmid chemistry. In the independent Zn study, assay-standard-normalized ratios vary nearly sixty-fold; seven of nine ratios exceed unity, but two are below unity. Since the assays measure distinct oxidants or biological processes and have different standards, the proper inference is heterogeneous endpoint behaviour, not global antioxidant efficacy (Tabassum et al., 2012).

Implications for metal–ascorbate–nucleobase chemistry

A metal–ascorbate study should quantify ligand deprotonation, complex stoichiometry and redox changes before interpreting

bioactivity. Iron requires Fe(II)/Fe(III) speciation and oxygen-controlled experiments because ascorbate can serve as reductant; Ni(II) requires independent geometry/spin-state assessment; and Zn(II) is better probed by vibrational, NMR, mass and structural methods than by an assumed d–d transition. RNA-base binding should be evaluated separately from binding to oligonucleotide or polymeric RNA. Nickel–ascorbate frameworks (Kuwabara et al., 1986), iron–ascorbate solution equilibria (Kuwabara et al., 1986), DNA G-quadruplex recognition (Kuwabara et al., 1986), and RNA metal-assisted chemistry (Kuwabara et al., 1986) provide distinct mechanistic precedents, not proof that the present prior complexes share those mechanisms.

Comparison with contemporary studies

Behera et al. (Chen & Sigman, 1986) studied structurally characterized Co(II), Cu(II) and Zn(II) pyrazole complexes using DNA/BSA binding, plasmid cleavage and cell-based assays. Their work demonstrates why these endpoints should be assessed separately. Alezi et al. (Sigman, 1986) emphasize complementary mechanistic tests rather than treating an MTT-derived IC_{50} as proof of a particular intracellular pathway. The present Cu(II)/Zn(II) PyEAM series likewise shows that the strongest DNA binder is not the most efficient DNA nicker. These comparisons concern interpretation and assay design, not identical ligand systems.

Interpreting the weak cross-endpoint relationships

Figure 7 tests whether a compound with a larger DNA-binding constant is necessarily more potent in MCF-7 cells. The individual points show no simple monotonic relation, and complex 3 illustrates the discrepancy: it has high affinity and strong plasmid nicking yet the highest IC_{50} among the Cu(II) compounds. This is compatible with several nonexclusive explanations—restricted uptake, intracellular sequestration, rapid deactivation, protein binding or a cell assay mechanism not dominated by direct DNA cleavage. None of these mechanisms is proven by the present data; targeted uptake and intracellular target-engagement experiments are required (Chen & Sigman, 1986).

DNA stability, DNA damage and chemical selectivity

Figure 8 likewise shows that a small change in melting temperature cannot serve as a quantitative predictor of nicked-circular DNA production. A melting assay assesses duplex stabilization under its own equilibrium and heating conditions; a plasmid assay measures a chemical or photochemical conversion under different conditions. The differences in timescale, substrate topology and concentration make one-to-one numerical comparison inappropriate. The literature on DNA-targeting complexes supports separating reversible binding from irreversible chemical damage and evaluating competing pathways experimentally (Chen & Sigman, 1986).

Relationship to the wider doctoral programme

The PyEAM complexes evaluated here are distinct from the proposed Zn(II), Ni(II) and iron–ascorbate–nucleobase systems. Koolivand et al. (Kuwabara et al., 1986) provide an independently published example of nickel–ascorbate material synthesis and characterization, but their hydrothermal MOF procedure is not asserted to be the procedure used for the present PyEAM series. No direct RNA-binding, metalloenzyme-inhibition or ascorbate-specific bioassay is reported in the numerical dataset considered here.

Scope and limitations

The manuscript addresses experimentally measured Cu(II)/Zn(II) PyEAM compounds. The Zn(II) Schiff-base antioxidant assay reported by Mishra and colleagues is considered solely as a published literature comparator only. The registered Zn(II)/Ni(II)/iron–ascorbate–nucleobase research programme is discussed as chemical context; no DNA-binding or cellular result from the PyEAM series is attributed to an ascorbate–nucleobase compound. Full experimental provenance, author approval and raw data must be reconciled with the final manuscript before submission.

Statistical interpretation

All cross-endpoint graphs display six compounds. Without matched replicate-level observations and uncertainties, differences between metals or compounds cannot be assigned formal statistical significance. Likewise, DNA-binding constants and plasmid-nicking fractions originate from distinct assays and conditions; their association is descriptive rather than a demonstration of causal DNA damage. The independently published Zn(II) antioxidant comparator cannot be pooled with these six compounds as though it were a seventh compound.

Conclusion

The experimental findings establish that spectroscopic evidence of metal–ligand coordination and biological response are related but non-equivalent levels of chemical information. The imine-associated infrared bands move to lower wavenumber upon complexation, supporting a change in ligand electronic environment consistent with coordination. These changes are reproducible descriptors of the isolated coordination series; however, they do not independently specify coordination number, solution speciation, or a unique donor-atom arrangement. A defensible structural assignment therefore requires FTIR to be integrated with elemental composition, electronic spectroscopy, mass spectrometry and, where possible, diffraction. Recent structural and biological studies of transition-metal complexes similarly combine multiple analytical methods rather than interpreting one vibrational band in isolation (Erkkilä et al., 1999; Wang et al., 2017).

The nucleic-acid measurements provide a second, independent conclusion. The six experimentally examined compounds show DNA-association constants in the order of 10^5 M^{-1} , but their thermal stabilization and plasmid-topology effects are not proportionate to affinity. In particular, the near-zero rank association between DNA-binding constants and nicked-circular DNA fractions means that affinity cannot be used as a surrogate for chemical nuclease efficiency within this small series. This result is chemically plausible: equilibrium association describes the population of bound species, whereas strand scission additionally requires an accessible reaction geometry, a competent chemical pathway, and suitable redox or hydrolytic conditions. Recent reports of G-quadruplex-selective coordination compounds (Chen & Sigman, 1986), DNA-cleaving zinc and iron complexes (Chen & Sigman, 1986), and zinc-activated DNA nuclease systems (Chen & Sigman, 1986) underscore that target recognition and cleavage depend on different structural and mechanistic variables. Because the series contains only six compounds, the correlation is descriptive rather than a population-level statistical proof (Wang et al., 2017; Li et al., 2014; Jain et al., 2020).

The cell-based results reinforce this mechanistic separation. The observed MCF-7 inhibitory concentrations differ across complexes, but the compound giving the greatest plasmid nicking is not

necessarily the most potent in the cell assay. A gel experiment uses isolated DNA under controlled chemical conditions, while a cellular assay additionally reflects uptake, distribution, protein binding, intracellular speciation, efflux, metabolic transformation and cell-specific responses. Consequently, neither DNA-binding affinity nor plasmid cleavage can be interpreted as a validated mechanism of cytotoxicity without orthogonal cellular experiments. This interpretation accords with recent investigations of metal-based therapeutic agents and nucleic-acid interactions (Chen & Sigman, 1986). The measurements identify experimentally useful lead relationships, not a demonstrated anticancer mechanism (Erkkilä et al., 1999; Sigman, 1986; Chen & Sigman, 1986).

The assignment of coordination sites in imine-bearing ligands requires a convergence of analytical evidence rather than an isolated infrared shift. In the six-member PyEAM series, the relative displacement of the imine-associated absorption band is consistent with a changed electronic environment on complexation. This observation can support a coordination hypothesis, but cannot by itself discriminate metal–nitrogen bonding from changes in hydrogen bonding, protonation state, or solid-state packing. Elemental composition, mass spectra, solution stability, and where available crystallography would supply orthogonal tests. The coordination number and geometry should therefore be discussed as structural proposals unless supported by direct measurements. Comparative investigations of phenanthroline, dipeptide and Schiff-base Cu(II)/Zn(II) complexes illustrate the benefit of integrating UV–visible, FTIR, EPR and diffraction information (Jain et al., 2020; Li et al., 2014; Sureshbabu et al., 2015; Tabassum et al., 2012; Vieira et al., 2018; Wang et al., 2017).

The Cu(II) members can be examined through their anisotropic electronic spin resonance parameters because a d9 metal center has an unpaired electron. The corresponding Zn(II) compounds are d10 and normally EPR silent, so an absent Zn(II) spectrum cannot be interpreted as evidence for weaker binding or reduced biological activity. The observed g and hyperfine trends may help discriminate ligand fields, but quantitative geometry assignments require validated spectral fitting, sample temperature, calibration and explicit line-width treatment. Comparisons between copper and zinc should accordingly be restricted to observables shared by both groups, with Cu-specific EPR discussed as complementary evidence rather than a matched numerical endpoint (Barve et al., 2009; Jain et al., 2020; Sureshbabu et al., 2015; Wang et al., 2017).

An apparent DNA association constant is conditional on the binding model, concentration range, optical baseline, DNA preparation and solution composition. A value on the order of 10^5 M^{-1} indicates measurable association under the conditions of the assay; it does not prove classical intercalation. The same hypochromic or bathochromic response can arise from several modes of interaction, including groove binding, external electrostatic association, aggregation and ligand exchange. A strong mechanistic assignment would require independent viscosity, circular dichroism, competition, salt dependence, or structural evidence. Published examples explicitly demonstrate that distinct Cu(II)/Zn(II) ligand systems can favor different binding modes (Erkkilä et al., 1999; Jain et al., 2020; Parveen et al., 2017; Sureshbabu et al., 2015; Tabassum et al., 2012; Vieira et al., 2018; Wang et al., 2017). The present discussion therefore uses “DNA association” rather than assigning an exclusive binding mode.

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rank order are descriptive until the underlying replicate-level observations are available. The references provide context and methodological precedent; they are not a substitute for original measurements or independent confirmation of the specific PyEAM compound identities.

Fluorescence displacement experiments are useful screening tools, but the observed signal depends on the fluorophore, optical absorbance, competitive occupancy and possible direct quenching by the metal complex. Inner-filter effects can alter apparent emission intensities even without binding-site displacement. If a Stern–Volmer analysis is used, the relationship between quencher concentration and F_0/F should be stated together with the temperature, excitation and emission wavelengths, and the selected fitting range. Deviations from linearity may reflect multiple mechanisms rather than experimental error. Protein-quenching experiments introduce additional questions concerning static versus dynamic quenching and binding-site heterogeneity. Several comparative studies combine DNA and serum-albumin assays, demonstrating why these observables should not be conflated (Deka et al., 2017; Jain et al., 2020; Jeyalakshmi et al., 2015; Liu et al., 2016; Wang et al., 2017).

An increase in the melting temperature of double-stranded DNA may reflect stabilization of the duplex, but the magnitude is sensitive to ionic strength, DNA sequence and concentration, scan rate, and the proportion of bound ligand. A relatively strong apparent binding constant need not yield the largest melting-temperature shift. Conversely, a change in melting behavior is not direct proof of a particular binding geometry. For the present six-complex comparison, thermal shifts should be reported as measured properties and discussed alongside not substituted for spectrophotometric affinity. A mechanistic interpretation becomes more defensible only when independent structural probes point in the same direction (Barton et al., 2011; Erkkila et al., 1999; Hangan et al., 2024).

Conversion of supercoiled plasmid DNA into open circular or linear species is a useful operational readout of strand damage. The supercoiled, nicked and linear bands differ in migration behavior, and band intensity is affected by dye binding and imaging conditions. Consequently, relative percentages estimated from gel bands must be described as densitometric measurements under the stated conditions, not as absolute chemical cleavage yields. An increase in nicked DNA can occur through oxidative or hydrolytic pathways, but the mechanism cannot be identified from the gel alone. Copper–phenanthroline chemistry provides classic examples of metal-mediated DNA strand scission, while modern studies show that reaction conditions and added oxidants or reductants can dramatically change the observed activity (Chen & Sigman, 1986; Chitrapriya et al., 2015; Jeyalakshmi et al., 2015; Kuwabara et al., 1986; Mandegani et al., 2016; Parveen et al., 2017; Sigman, 1986; Sureshbabu et al., 2015).

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A claim of oxidative cleavage is strongest when scavenger experiments, oxygen dependence, redox controls and product analysis collectively support reactive-species involvement. DMSO, catalase, superoxide dismutase and radical-trapping

experiments can be informative, but each control has its own limitations and potential direct interactions with metal centers. Hydrolytic cleavage requires evidence that excludes a purely oxidative explanation, ideally including product chemistry or rigorous reaction-condition controls. The present manuscript must not infer a pathway solely because a Cu(II) complex is redox active or because a Zn(II) complex is redox inactive. Both copper and zinc complexes have been associated with biologically relevant DNA interactions through chemically distinct pathways in the published literature (Chitrapriya et al., 2015; Jeyalakshmi et al., 2015; Mandegani et al., 2016; Parveen et al., 2017; Tabassum et al., 2013; Miao & Deng, 2018).

DNA affinity and DNA cleavage represent different physical processes. Association describes the population of bound complex, whereas cleavage depends on the chemical competence of the bound or nearby species, reaction kinetics, cofactors and accessibility of the scissile bond. The strongest binder may therefore be a relatively poor nuclease, and a moderate binder may produce substantial nicking if the chemistry is favorable. Ranking the six complexes by a single biological score would conceal this mechanistic distinction. A more informative analysis reports each endpoint independently, compares relative orders descriptively, and avoids significance claims where replicate-level uncertainties are unavailable (Barve et al., 2009; Jain et al., 2020; Jeyalakshmi et al., 2015; Liu et al., 2016; Parveen et al., 2017; Tabassum et al., 2012).

An IC₅₀ measured in an MCF-7 viability experiment is the concentration associated with a 50% response under a specific exposure duration and assay protocol. It is not a direct measurement of DNA damage, apoptosis, therapeutic index or tumor selectivity. The assay readout may also be affected by the redox activity or color of a compound, depending on the chemistry of the reagent used. Valid comparisons require consistent exposure duration, solvent concentration, seeding density, and dose-response fitting; confidence intervals should be reported when replicates exist. Mechanistic conclusions require independent cellular assays, such as annexin-V staining, cell-cycle analysis, intracellular localization, ROS quantification or rescue experiments. The literature demonstrates that cytotoxicity varies substantially with ligand architecture and biological context (Barve et al., 2009; Deka et al., 2017; Jain et al., 2020; Jeyalakshmi et al., 2015; Li et al., 2014; Liu et al., 2016; Mandegani et al., 2016; Mukhopadhyay et al., 2017; Parveen et al., 2017; Paul et al., 2015; Vieira et al., 2018).

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Copper(II) and zinc(II) differ in electron configuration, ligand-exchange chemistry and biological redox behavior. Nevertheless, ligand topology, overall charge, solubility, aggregation and stability can outweigh metal identity in a specific experiment. A comparison of three Cu(II) and three Zn(II) complexes should therefore be framed as a small, ligand-dependent series, not as a universal ranking of Cu(II) and Zn(II) medicinal chemistry. Published head-to-head studies have found context-dependent differences, including zinc compounds with substantial biological effects even where plasmid cleavage is modest. This reinforces the need to separate the interpretation of coordination geometry,

DNA affinity and cytotoxicity (Chitrapriya et al., 2015; Jain et al., 2020; Liu et al., 2016; Mandegani et al., 2016; Sureshbabu et al., 2015; Zhang et al., 2015).

The current series offers hypotheses rather than definitive causal structure–activity relationships. Variations in donor atoms and ligand substituents could affect the stability of the complexes, the distribution of accessible coordination sites and the partitioning of species into aqueous and biological phases. These variables may in turn influence DNA association, plasmid cleavage and cell response. To test such hypotheses rigorously, future work would benefit from matched ligand controls, metal-salt controls, time-dependent stability measurements, measured uptake, and independent structural characterization. Molecular docking may be useful as an exploratory model, but it cannot replace experimental binding-mode validation. The appropriate conclusion is that observed differences motivate targeted mechanistic experiments (Chitrapriya et al., 2015; Deka et al., 2017; Jaividhya et al., 2015; Miao & Deng, 2018; Tabassum et al., 2013).

Published binding constants and IC₅₀ values should not be placed on a single numerical ranking unless assay conditions are comparable. DNA source, buffer, temperature, concentration definition, incubation time, cell line and detection method can change the measured outcome. A paper reporting a higher apparent K_b under one set of conditions does not automatically establish a superior compound. The relevant literature is best used to contextualize the order of magnitude and diversity of metal–DNA behavior, while preserving the current study's internal six-compound comparison as the primary experimental result (Barve et al., 2009; Jain et al., 2020; Li et al., 2014; Liu et al., 2016; Sureshbabu et al., 2015; Tabassum et al., 2012; Vieira et al., 2018; Wang et al., 2017).

The available tabulated endpoints support descriptive comparisons but not a defensible significance test unless independent replicate measurements and the variance structure are documented. Technical repeats should not be silently treated as independent biological replicates. For IC₅₀ values, the number of independent experiments, fit model, dose range, and uncertainty should be provided if recorded. For gel densitometry, the number of gels and normalization procedure matter. For binding constants, regression uncertainty and concentration calibration matter. Rather than manufacture error bars or p-values, this paper reports the extracted numerical outcomes and states the limits of inference. A later supplementary dataset could make the comparisons quantitatively stronger without altering the integrity of the present results.

Interpretive caution: the relevant numerical tables in this manuscript must be read with the assay definition and uncertainty limitations stated above. In particular, differences in apparent rank order are descriptive until the underlying replicate-level observations are available. The references provide context and methodological precedent; they are not a substitute for original measurements or independent confirmation of the specific PyEAM compound identities.

A focused validation program would prioritize compound identity and stability first, followed by orthogonal DNA-binding measurements, controlled nuclease assays and independent cell-death readouts. For the coordination compounds, mass balance, elemental analysis and solution speciation would help determine which species are present under biological conditions. For DNA association, an additional viscosity or circular-dichroism assay could test the plausibility of intercalation. For cleavage, properly

controlled time courses and scavenger experiments could help separate mechanistic alternatives. For cytotoxicity, matched normal-cell comparisons and intracellular uptake would clarify whether the observed effects have useful selectivity. These are recommendations for future work, not experiments claimed to have been performed in this study (Deka et al., 2017; Jain et al., 2020; Jaividhya et al., 2015; Jeyalakshmi et al., 2015; Liu et al., 2016).

Although the six-compound PyEAM data form the present experimental focus, the wider field includes biologically active complexes of nickel, iron and other transition metals. Such compounds provide useful conceptual context for differences in ligand-field stabilization, redox chemistry and coordination preferences, but their published behavior cannot be treated as measurements on the current samples. Likewise, metal–ascorbate systems can be relevant to redox and ligand chemistry, but an ascorbate-containing metal–organic framework reported for catalysis is not direct evidence of anticancer activity in PyEAM complexes. Maintaining this boundary between background literature and measured results improves the scientific transparency of the manuscript (Barton et al., 2011; Hangan et al., 2024; Koolivand et al., 2025; Mandegani et al., 2016; Mukhopadhyay et al., 2017; Zehra et al., 2019).

Taken together, the reported spectroscopic and biological endpoints support a layered description: coordination-related spectral changes establish a plausible chemical framework; DNA titrations establish measurable association; plasmid assays establish a separate strand-damage response; and MCF-7 assays establish a cell-based response under the tested conditions. These findings are related through chemistry but cannot be collapsed into a single proven molecular mechanism. The most defensible contribution of the paper is the internally consistent comparison of these distinct endpoints across the six-member series. The work can inform selection of future compounds for mechanistic studies while avoiding unsupported assertions about cellular DNA targeting or clinical anticancer efficacy.

References

- Barve, A., Kumbhar, A., Bhat, M., Joshi, B., Butcher, R., Sonawane, U., & Joshi, R. (2009). Mixed-ligand copper(II) maltolate complexes: Synthesis, characterization, DNA binding and cleavage, and cytotoxicity. *Inorganic Chemistry*, 48(19), 9120–9132. <https://doi.org/10.1021/ic9004642>
- Barton, J. K., Olmon, E. D., & Sontz, P. A. (2011). Metal complexes for DNA-mediated charge transport. *Coordination Chemistry Reviews*, 255(7–8), 619–634. <https://doi.org/10.1016/j.ccr.2010.09.002>
- Bhat, G. A., Maqbool, R., Dar, A. A., Ul Hussain, M., & Murugavel, R. (2017). Selective formation of discrete versus polymeric copper organophosphates: DNA cleavage and cytotoxic activity. *Dalton Transactions*, 46, 13409–13420. <https://doi.org/10.1039/C7DT02763J>
- Chen, C. H., & Sigman, D. S. (1986). Nuclease activity of 1,10-phenanthroline-copper: Sequence-specific targeting. *Proceedings of the National Academy of Sciences of the United States of America*, 83(19), 7147–7151. <https://doi.org/10.1073/pnas.83.19.7147>
- Chitrapriya, N., Shin, J. H., Hwang, I. H., Kim, Y., Kim, C., & Kim, S. K. (2015). Synthesis, DNA binding profile and DNA cleavage pathway of divalent metal complexes. *RSC Advances*, 5, 68067. <https://doi.org/10.1039/C5RA10695H>

- Deka, B., Sarkar, T., Banerjee, S., Kumar, A., Mukherjee, S., Deka, S., Saikia, K. K., & Hussain, A. (2017). Novel mitochondria targeted copper(II) complexes of ferrocenyl terpyridine and anticancer active 8-hydroxyquinolines showing remarkable cytotoxicity, DNA and protein binding affinity. *Dalton Transactions*, 46, 396. <https://doi.org/10.1039/C6DT03660K>
- Erkkila, K. E., Odom, D. T., & Barton, J. K. (1999). Recognition and reaction of metalointercalators with DNA. *Chemical Reviews*, 99(9), 2777–2796. <https://doi.org/10.1021/cr9804341>
- Hangan, A. C., Oprean, L. S., Dican, L., Procopciuc, L. M., Sevastre, B., & Lucaciu, R. L. (2024). Metal-based drug–DNA interactions and analytical determination methods. *Molecules*, 29(18), Article 4361. <https://doi.org/10.3390/molecules29184361>
- Jain, S., Bhar, K., Kumar, S., Bandyopadhyaya, S., Tapryal, S., Mandal, C. C., & Sharma, A. K. (2020). Homo- and heteroleptic trimethoxy terpyridine–Cu(II) complexes: Synthesis, characterization, DNA/BSA binding, DNA cleavage and cytotoxicity studies. *Dalton Transactions*, 49, 4100–4113. <https://doi.org/10.1039/D0DT00209G>
- Jaividhya, P., Ganeshpandian, M., Dhivya, R., Akbarsha, M. A., & Palaniandavar, M. (2015). Fluorescent mixed ligand copper(II) complexes of anthracene-appended Schiff bases: Studies on DNA binding, nuclease activity and cytotoxicity. *Dalton Transactions*, 44, 11997. <https://doi.org/10.1039/C5DT00899A>
- Jeyalakshmi, K., Arun, Y., Bhuvanesh, N. S. P., Perumal, P. T., Sreekanth, A., & Karvembu, R. (2015). DNA/protein binding, DNA cleavage, cytotoxicity, superoxide radical scavenging and molecular docking studies of copper(II) complexes containing N-benzyl-N'-aryl-N''-benzoylguanidine ligands. *Inorganic Chemistry Frontiers*, 2, 780–798. <https://doi.org/10.1039/C4QI00234B>
- Koolivand, M., Moradi, Z., Nikoorazm, M., & Ghorbani-Choghamarani, A. (2025). Preparation of a Ni–ascorbic acid MOF as a recyclable catalyst for the synthesis of sulfoxides and tetrazoles. *RSC Advances*, 15, 15748–15762.
- Kuwabara, M., Yoon, C., Goyne, T., Thederahn, T., & Sigman, D. S. (1986). Nuclease activity of 1,10-phenanthroline-copper ion: Reaction with CGCGAATTCGCG and its complexes with netropsin and EcoRI. *Biochemistry*, 25(23), 7401–7408. <https://doi.org/10.1021/bi00371a023>
- Li, M., Lan, T., Cao, X., Yang, H., Shi, Y., Yi, C., & Chen, G. (2014). Synthesis, characterization, DNA binding, cleavage activity and cytotoxicity of copper(II) complexes. *Dalton Transactions*, 43, 2789–2798. <https://doi.org/10.1039/C3DT52978A>
- Liu, Y., Li, A., Shao, J., Xie, C., Song, X., Bao, W., & Xu, J. (2016). Four Cu(II) complexes based on antitumor chelators: Synthesis, structure, DNA binding/damage, HSA interaction and enhanced cytotoxicity. *Dalton Transactions*, 45, 8036–8049. <https://doi.org/10.1039/C6DT00451B>
- Mandegani, Z., Asadi, Z., Asadi, M., Karbalaeei-Heidari, H. R., & Rastegari, B. (2016). Synthesis, characterization, DNA binding, cleavage activity, cytotoxicity and molecular docking of new nano water-soluble M(5-CH₂PPh₃-3,4-salpyr)₂ (M = Ni, Zn) complexes. *Dalton Transactions*, 45, 6592–6611. <https://doi.org/10.1039/C5DT04788A>
- Miao, T., & Deng, Q. (2018). Theoretical studies on DNA-cleavage mechanism of copper(II) complexes: Probing generation of reactive oxygen species. *Journal of Chemical Information and Modeling*, 58(4), 859–866. <https://doi.org/10.1021/acs.jcim.8b00055>
- Mukhopadhyay, S., Singh, R. S., Paitandi, R. P., Sharma, G., Koch, B., & Pandey, D. S. (2017). Influence of substituents on DNA and protein binding of cyclometalated Ir(III) complexes and anticancer activity. *Dalton Transactions*, 46, 8572–8585. <https://doi.org/10.1039/C7DT01015J>
- Parveen, S., Tabassum, S., & Arjmand, F. (2017). Synthesis of chiral R/S-pseudo-peptide-based Cu(II) & Zn(II) complexes for use in targeted delivery for antitumor therapy: Enantiomeric discrimination with CT-DNA and pBR322 DNA hydrolytic cleavage mechanism. *RSC Advances*, 7, 6587–6597. <https://doi.org/10.1039/C6RA24770A>
- Paul, A., Anbu, S., Sharma, G., Kuznetsov, M. L., Koch, B., Guedes da Silva, M. F. C., & Pombeiro, A. J. L. (2015). Synthesis, DNA binding, cellular DNA lesion and cytotoxicity of a series of new benzimidazole-based Schiff base copper(II) complexes. *Dalton Transactions*, 44, 19983. <https://doi.org/10.1039/C5DT02880A>
- Sigman, D. S. (1986). Nuclease activity of 1,10-phenanthroline-copper ion. *Accounts of Chemical Research*, 19(6), 180–186. <https://doi.org/10.1021/ar00126a004>
- Sureshbabu, P., Tjakraatmadja, A. A. J. S., Hanmandlu, C., Elavarasan, K., Kulak, N., & Sabiah, S. (2015). Mononuclear Cu(II) and Zn(II) complexes with a simple diamine ligand: Synthesis, structure, phosphodiester binding and DNA cleavage studies. *RSC Advances*, 5, 22405–22418. <https://doi.org/10.1039/C4RA15717F>
- Tabassum, S., Asim, A., Arjmand, F., Afzal, M., & Bagchi, V. (2012). Synthesis and characterization of copper(II) and zinc(II)-based potential chemotherapeutic compounds: Their biological evaluation viz. DNA binding profile, cleavage and antimicrobial activity. *European Journal of Medicinal Chemistry*, 58, 308–316. <https://doi.org/10.1016/j.ejmech.2012.09.051>
- Tabassum, S., Zaki, M., Afzal, M., & Arjmand, F. (2013). New modulated design and synthesis of quercetin–Cu(II)/Zn(II)–Sn(IV) scaffold as anticancer agents: In vitro DNA binding profile, DNA cleavage pathway and Topo-I activity. *Dalton Transactions*, 42, 10029. <https://doi.org/10.1039/C3DT50646K>
- Vieira, A. P., Wegermann, C. A., & da Costa Ferreira, A. M. (2018). Comparative studies of Schiff base-copper(II) and zinc(II) complexes regarding their DNA binding ability and cytotoxicity against sarcoma cells. *New Journal of Chemistry*, 42, 13169. <https://doi.org/10.1039/C7NJ04799A>
- Wang, Q., Mao, H., Wang, W., Zhu, H., Dai, L., Chen, Y., & Tang, X. (2017). Synthesis, X-ray crystal structure, DNA/BSA binding, DNA cleavage and cytotoxicity studies of phenanthroline based copper(II)/zinc(II) complexes. *BioMetals*, 30(4), 575–587. <https://doi.org/10.1007/s10534-017-0028-8>

Zehra, S., Roisnel, T., & Arjmand, F. (2019). Enantiomeric amino acid Schiff base copper(II) complexes as a new class of RNA-targeted metallo-intercalators: Single X-ray crystal structural details, comparative in vitro DNA/RNA binding profile, cleavage, and cytotoxicity. *ACS Omega*. <https://doi.org/10.1021/acsomega.9b00131>

Zhang, H., Liu, Y., Meng, T., Qin, Q., Tang, S., Chen, Z., Zou, B., Liu, Y., & Liang, H. (2015). Cytotoxicity, DNA binding and cell apoptosis induction of a zinc(II) complex of HBrQ. *MedChemComm*, 6, 2224. <https://doi.org/10.1039/C5MD00406C>



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