Synthesis and Characterization of Pyridine-Based Ternary Metal Complexes with Anticancer and Antimicrobial Activities

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Abstract

The search for novel anticancer agents with minimal reliance on platinum is crucial. This study explored the potential of newly synthesized pyridine-based mixed ligand metal complexes. Characterized by physicochemical and multispectral methods, the mixed ligand complexes exhibit an octahedral geometry. The complexes are suggested to bind DNA through intercalation, evident from absorption bands in the 330-350 nm region accompanied by 19-28% bathochromic and hypochromic effects. Docking simulations were employed to substantiate further the proposed intercalative binding mode of the synthesized compounds with nucleic acids. Notably, the copper complex displayed superior efficacy, with lower minimum inhibitory concentrations (MICs) against bacteria and fungi (8-10 x 10^4 μM). Highlighting its potential as a therapeutic agent, the copper complex displayed exceptional efficacy against human cancer cell lines. This is evident from its deficient half-maximal inhibitory concentration (IC_{50}) values, indicating potent cytotoxicity. The copper complex effectively inhibited the growth of MCF-7 and Hep G2 cancer cells with IC_{50} values of 16 μM and 17 μM, respectively. Furthermore, the metal complexes are predicted to possess higher antioxidant activity than free ligand, and insilico ADMET evaluation suggested favorable drug-like properties. These findings highlighted the copper complex as a promising candidate for further development as a novel anticancer agent.

Keywords: Mixed ligand complexes, Intercalation; Cytotoxicity; Antioxidant activity; Insilico ADMET; Docking simulations.

1. Introduction

Ternary metal complexes, containing a central metal ion coordinated with two different ligands, are valuable models for understanding metal-enzyme-substrate interactions in biological systems [1]. These complexes can mimic the behaviour of metalloenzymes, where mixed-ligand interactions and covalent bond formation between ligands play a crucial role in

stabilizing the enzyme-substrate complex and contributing to the enzyme's specificity [2]. Research on ternary complexes, particularly those involving aromatic Schiff bases and 1,10-phenanthroline, has provided valuable insights into these mechanisms [3]. Understanding how metal-based drugs interact with nucleic acids such as DNA is paramount in developing new and effective anticancer agents [4, 5]. The efficacy of these interactions hinges on the chosen strategy and the strength of the binding between the drug and the DNA target. Therefore, studying metal complexes and their interactions with DNA is crucial for developing and improving chemotherapy medications. Transition metal complexes with the ability to bind and cleave DNA under specific conditions have garnered significant interest in nucleic acid chemistry due to their potential therapeutic applications [6-12].

Pyridine, a six-membered aromatic ring containing a nitrogen atom, serves as a versatile building block in various chemical applications. Its ability to coordinate with diverse metal ions allows for the formation of a wide range of metal complexes. Pyridine-based metal complexes can function as electrochemical or colorimetric sensors, detecting specific molecules through changes in their electrical or optical properties [13]. Organometallic catalysts containing pyridine or its derivatives play a crucial role in promoting organic reactions. Pyridine and related compounds are particularly significant in the pharmaceutical field due to their potent biological activity [14,15]. This characteristic makes them valuable starting points for drug development.

Pyridine, a nitrogen-containing aromatic heterocycle, has emerged as a linchpin in drug discovery due to its frequent presence in a wide range of medications, including antibacterial, antiviral, antihistamine, and anticancer drugs. Antibacterial, antiviral, antihistamine, and anticancer drugs often incorporate this core structure. This widespread application stems from pyridine's remarkable ability to bind to a variety of biomolecules critical for cellular function, including proteins, DNA, coenzymes, amino acids, and metabolites [16]. Pyridine's therapeutic potential stems from its unique confluence of properties. Its basicity allows it to accept protons, facilitating interactions with targeted biomolecules. High water solubility allows for effective drug absorption and distribution throughout the body. Furthermore, the rigidity and aromaticity of the pyridine ring endow it with stability, crucial for maintaining its shape and facilitating effective drug-target interactions. Additionally, pyridine's ability to participate in hydrogen bonding further enhances its binding affinity to biomolecules. Finally, the small molecular size of pyridine promotes membrane permeability, aiding in drug absorption and maximizing its therapeutic effect.

The pyridine ring, a key building block in many bioactive molecules, finds frequent application in drugs for bacterial infections, viruses, allergies, and even cancer. This popularity stems from pyridine's remarkable ability to bind with essential biomolecules like proteins, DNA, coenzymes, amino acids, and metabolites. In drug discovery, strategically replacing functional groups like amines, amides, and heterocycles with nitrogen atoms, and benzene rings with pyridine moieties can be a powerful strategy [17,18]. This approach capitalizes on bioisosterism, where pyridine mimics the behaviour of these groups while potentially offering improved properties.

This study explores the development of novel therapeutic agents through the synthesis of mixed-ligand metal complexes. We reported the creation of complexes by combining a newly synthesized Schiff base ligand derived from 3-Amino-4-hydroxypyridine and 10-Chloro-9-anthraldehyde with 1,10-phenanthroline (co-ligand) and various transition metals (Cu(II), Co(II), Ni(II), and Zn(II)). The synthesized complexes demonstrated a compelling combination of properties, including efficient DNA binding, antimicrobial efficacy and free radical scavenging potential.

2. Experimental

2.1. Synthesis of Schiff base ligand

To synthesize the Schiff base ligand (Scheme 1), a solution of 10-chloro-9-anthraldehyde (2.406 g/mol, 10 mmol) and 3-amino-4-hydroxypyridine (1.1012 g/mol, 10 mmol) in ethanol (1.1011 g, 10 mmol) were prepared. A few drops of acetic acid and K_2CO_3 were added and the mixture was refluxed in a water bath for 8 hours. After cooling, the reaction mixture was poured onto crushed ice. The yellow solid was filtered and purified by recrystallization in ethanol.

Yield: 89%. Schiff base ligand (L); $C_{20}H_{13}ClN_2O$; Colour: Yellow; Anal. cal. for ligand C 72.18%, H 3.94%, N 8.42%; Found: C 71.3%, H 3.84%, N 8.32%; FT-IR (KBr disc cm^{-1}): 3610 ν(-OH), 1584 ν(-CH=N), ^{1}H NMR (DMSO-d_6) (δ): (aromatic) 6.92-8.55 (m), (C-CH3), (-CH=N) 8.71 (s); (-OH) 9.91 (s); ^{13}C NMR (DMSO-*d6*) (δ): (aromatic) 113.78-145.39 (m), (-CH=N) 151.95 (s), (C-OH) 162.48 (s), ESI-MS: 332 (Molecular ion peak).

2.2. Synthesis of mixed ligand complexes

20 millimoles (mmol) each of the Schiff base ligand (6.64 g) and a metal chloride salt, along with 10 mmol of the co-ligand 1,10-phenanthroline (1.80 g), were dissolved in a

suitable amount of ethanol (molar ratio 2:1:1). The mixture was sonicated for 8 hours, followed by refluxing with stirring for 24 hours. The resulting product was recrystallized in ethanol and dried under vacuum over anhydrous $CaCl_2$ (Scheme 2).

$C_{52}H_{32}Cl_2N_6O_2Cu$; Yield: 82%; M. Wt: 905; Colour: Brown; Anal. cal. for Cu(II) Complex C 68.84 %, H 3.56 %, N 9.26 %, Cu 7.00 %; Found: C 68.24%, H 3.53 %, N 9.21%, Cu 6.89 %; $\Lambda m \times 10^{-3}(\Omega^{-1}mol^{-1}cm^{-2})$18; BM:1.79; λ_{max} nm in DMSO 660 (d-d); FT-IR (KBr disc cm^{-1}): ν(-CH=N) ; 1584; 548 (M-O), 432 (M-N) cm^1; ESI-MS: 906 m/z (M+1).

$C_{52}H_{32}Cl_2N_6O_2Co$ Yield: 79%; M. Wt: 902; Colour: Pink; Anal. cal. for Co(II) Complex C 69.19 %, H 3.57 %, N 9.31 %, Co 6.53 %; Found: C 69.12 %, H 3.53%, N9.28%, Co 6.49 %; $\Lambda_m \times 10^{-3}(\Omega^{-1}mol^{-1}cm^{-2})$ 23; BM:4.23; λmax nm in DMSO 636 (d-d); FT-IR (KBr disc cm^{-1}): ν(-CH=N) ; 1578; 526 (M-O),438 (M-N) cm^1.

$C_{52}H_{32}Cl_2N_6O_2Ni$; Yield: 80%; M. Wt: 902; Colour: Yellowish green; Anal. cal. for Ni(II) Complex C 69.21 %, H 3.57 %, N 9.31 %, Ni 6.51 %; Found: C 69.11%, H 3.51 %, N 9.29 %, Ni 6.48 %; $\Lambda m \times 10^{-3}$ (Ω^{-1} mol^{-1} cm^{-2}) 28 ; BM: 3.26; λmax nm in DMSO 642 (d-d); FT-IR (KBr disc cm^{-1}): 1592 ν(-CH=N),548(M-O),434(M-N)cm^{-1}.

$C_{52}H_{32}Cl_2N_6O_2Zn$; Yield: 81 %; M. Wt: 909 ; Colour: Pale yellow; Anal. cal. for Zn(II) complex C 68.70%; H 3.55 %, N 9.24 %, Zn 7.19 %; Found: C 68.5 %, H 3.48%, N 9.23%, Zn 7.18 %, $\Lambda m \times 10^{-3}$ (Ω^{-1} mol^{-1} cm^{-2}) 21; μ_{eff} diamagnetic; λ_{max} nm in DMSO 354; FT-IR (KBr disc cm^{-1}): 1596 ν(-CH=N), 548 (M-O), 439 (M-N) cm^{-1}. ^{1}H NMR (DMSO-*d6*) (δ): (aromatic H) 6.91-8.55 (m), (-CH=N) 9.14 (s), ^{13}C NMR (DMSO-*d6*) (δ): (aromatic C) 113.78 - 145.39 (m), (-CH=N) 155.23 (s), (C=O) 156.72 (s).

3. Materials and Methods

In this research work, the reactants of chemicals were used as AnalaR grade without further purification. However, the solvent is used as a purified solvent by distillation method. The reagents 10-Chloro-9-anthraldehyde, 3-Amino-4-hydroxypyridine and 1,10-Phenanthroline were procured from Sigma Aldrich India. All other metal chloride salts were collected from Merck products.

4. Results and discussion

This study described the synthesis and characterization of novel metal complexes derived from a pyridine-based Schiff base ligand (SL). The ligand was prepared by condensation of 10-chloro-9-anthraldehyde with 3-amino-4-hydroxypyridine. Subsequently, the ligand was

reacted with metal chlorides in the presence of 1,10-Phenanthroline to yield mixed-ligand complexes with the proposed formula. The synthesized ligand and metal complexes are insoluble in water. Metal complexes exhibited good solubility in Dimethyl sulfoxide (DMSO) and dimethylformamide (DMF), while the ligand is soluble in common organic solvents. Conductivity measurements indicated that the complexes are non-electrolytes. The synthesis of the pyridine-based Schiff base ligand (SL) is outlined in Scheme 1. Scheme 2 subsequently illustrated the formation of the mixed-ligand metal complexes with the proposed formula $[M(L_2)(phen)]$.

4.1. FT-IR spectra

Fourier-transform infrared (FT-IR) spectroscopy provided valuable insights into the complexation process. Compared to the spectrum of the free ligand (Fig. S1), the spectra of the metal complexes (Fig. S2) revealed significant changes indicative of chelation [19]. The broad band observed at 3610 cm^{-1} in the ligand spectrum, assigned to the hydroxyl (-OH) group, disappeared in the complex spectra. This disappearance suggested the coordination of the (-OH) group with the metal ion [20]. Additionally, the strong band at 1584 cm^{-1}, attributed to the azomethine group vibration in the free ligand, shifted to a range of 1576-1598 cm^{-1} in all complexes. This shift indicated chelation of the azomethine nitrogen atom to the metal center [21, 22]. The emergence of two new bands in the low-frequency regions of the complex spectra further supported chelation. These bands, located at 420-440 cm^{-1} and 525-550 cm^{-1}, are assigned to υ(M-N) and υ(M-O) vibrations, respectively [23]. This observation confirmed the involvement of the azomethine nitrogen atom, co-ligand atoms, and the oxygen atom from the -OH group in complex formation.

4.2. Electronic spectra and magnetic moments

The geometries of the synthesized metal complexes were investigated using UV-visible absorption spectroscopy and magnetic susceptibility measurements. The free ligand exhibited two distinct absorption bands at 232 nm and 342 nm, assigned to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ respectively [24]. Coordination with metal ions altered the electronic environment around the chromophore, leading to a shift in the energies of these transitions for all complexes compared to the free ligand. The d-d band positions in the electronic spectra provided valuable information about the metal center geometries. The Cu(II) complex displayed a band at 660 nm, characteristic of the $^2E_g \rightarrow {}^2T_{2g}$ transition in a slightly distorted octahedral geometry. Similarly, the Co(II) complex exhibited a d-d band at 636 nm, consistent with the $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ transition expected for an octahedral arrangement. This assignment is

further supported by the observed magnetic susceptibility value of 4.23 BM for the Co(II) complex [25]. Absorption spectroscopy data revealed a d-d band at 642 nm, which is consistent with the $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ transition in octahedral Ni(II) complexes. Although spectroscopic data strongly suggested an octahedral geometry for the Cu(II), Co(II), and Ni(II) complexes, definitive confirmation through single-crystal X-ray diffraction (XRD) analysis was not possible due to limitations in growing crystals suitable for this technique. The electronic spectrum of ligand and Cu(II) complex are represented in Figs S3 and S4 respectively.

4.3. ^{1}HNMR and ^{13}CNMR

^{1}H NMR spectroscopy provided valuable insights into the coordination environment of the Zn(II) complex (Figs S5 and S6). The spectrum of the free Schiff base ligand (SL) displayed a multiplet at 6.92–8.55 ppm, assigned to the aromatic protons of the phenyl ring. Additionally, a singlet at 8.71 ppm was attributed to the azomethine (C=N) proton, and another singlet at 9.91 ppm corresponded to the hydroxyl (-OH) group on the pyridine moiety [26, 27].

Upon complexation with Zn(II), distinct changes were observed in the ^{1}H NMR spectrum, particularly for the -OH and -CH=N proton signals. These changes suggested the deprotonation of the hydroxyl group and chelation of the azomethine nitrogen with the Zn(II) ion [28]. Conversely, the aromatic proton signals remained largely unaffected, indicating minimal interaction between the metal center and the phenyl ring.

The analysis of the ^{13}C NMR spectra (Figs S7 and S8) strengthened the evidence for complex formation. The ligand (SL) exhibited signals for the phenyl carbons (113.78-145.39 ppm) and the azomethine carbon (151.95 ppm). In the Zn(II) complex spectrum, the azomethine carbon signal shifted to 155.23 ppm, indicating coordination with the metal ion. Additionally, the peak for the hydroxyl group carbon (-C-OH) at 162.48 ppm in the ligand disappeared in the complex. This disappearance is accompanied by the emergence of new peaks at 156.72 ppm, strongly suggesting the formation of a deprotonated carbonyl group (-C=O) that chelated the metal ion [29]. The absence of further significant changes in the Zn(II) complex spectrum reinforced the notion that the primary interaction involved the deprotonated hydroxyl group.

4.4. Electron Paramagnetic Resonance spectrum (EPR)

Electron paramagnetic resonance (EPR) spectroscopy is a valuable tool for analyzing the geometry, metal ion environment, and covalency of metal-ligand (M-L) bonds in copper (Cu(II)) complexes. The EPR spectrum (Fig. S9) provided information through the positions and spacing of its hyperfine lines. From the EPR spectrum, two key parameters are extracted. g-values are proportionality factors calculated using the equation $g = \upsilon / (B \times \gamma)$, where υ is the microwave frequency (X-band in this case), B is the magnetic field strength, and γ is the gyromagnetic ratio of the electron. The g-values reflected the electronic environment around the Cu(II) ion [30]. The nuclear hyperfine coupling constant (A) parameter indicated the interaction between the unpaired electron and the copper metal. The analysis provided information on both the parallel and perpendicular components of the data under investigation. Table 1 summarizes the EPR data (g-values and A) for the Cu(II) complex under study.

The observed values, $A_{\parallel}$ (168 G), $A_{\perp}$ (69 G), $g_{\parallel}$ (2.31), $g_{\perp}$ (2.07), and g_e (2.0023), suggested that the Cu(II) complex exhibited an octahedral geometry with a slight distortion. This is supported by the $g_{\parallel}/A_{\parallel}$ value of 137, which falls within the range expected for octahedral Cu(II) complexes [31]. The observed g_{iso} value deviated from the value expected for a purely ionic interaction (around 2.3), suggesting a covalent contribution to the M-L bond. The g-factor and G-value can also be used to evaluate potential exchange interactions between Cu(II) centers within the polycrystalline sample. A G-value lower than 4 would signify significant interaction between neighboring copper ions. Conversely, a G-value exceeding 4 could be indicative of either a slightly distorted or parallel local tetrahedral geometry around the Cu(II) centers, as reported previously [32]. The geometric parameter value obtained in this study (5.3 for Cu(II)) confirmed the monomeric nature of the complex, implying the absence of metal-metal (M-M) bonds involving the Cu(II) center. Fig. S9 displayed the actual EPR spectrum of the Cu(II) complex.

4.5. ESI-MS spectrum

Electrospray ionization mass spectrometry (ESI-MS) was employed to characterize the ligand (SL) and its copper(II) complex Figs. S10 and S11, respectively. The analysis of molecular ion peaks and fragmentation patterns provided valuable information about the complex's stoichiometry and potential geometry. The ligand spectrum exhibited a molecular ion peak at m/z 332, corresponding to the formula $[C_{20}H_{13}ClN_2O]$. This confirmed the successful synthesis of the ligand with the expected molecular weight. The spectrum of the ligand (Fig. S10) revealed several fragment ion peaks alongside the molecular ion peak at m/z

332. Moreover, the ligand has fragmental ion peaks at m/z 66, 78, 150, 164, 212, 239, 280, 304, 316 and 333 for probable $[C_5H_6]^+$, $[C_6H_6]^+$, $[C_9H_9Cl]^+$, $[C_{10}H_9Cl]^+$, $[C_{14}H_9O]^+$, $[C_{15}H_{10}ClN]^+$, $[C_{17}H_{13}ClN_2]^+$, $[C_{19}H_{13}ClN_2]^+$, $[C_{20}H_{13}ClN_2]^+$ and $[C_{20}H_{13}ClN_2O]^+$ fragmental ions respectively.

The ESI-MS spectrum of the Cu(II) complex (Fig. S11) displayed a crucial peak at m/z 906 (M+1), indicating the complex ion with one additional proton. This suggested a 1:2 (metal:ligand) stoichiometry in the complex. Based on the observed molecular weight and considering the known mass of the ligand, the tentative formula for the complex can be assigned as $[M(L_2)(Phen)]$, where M represents the copper(II) ion, L represents the ligand, and phen denoted 1,10-phenanthroline. The proposed structure is further supported by mass spectral data obtained for analogous complexes.

4.6. FE-SEM/EDX

Scanning electron microscopy (SEM) images (Fig. 5) of the Cu(II), Co(II), Ni(II), and Zn(II) complexes revealed diverse morphologies with smooth and rough surface zones. These observations supported the successful chelation of the metal ions within the complexes [33]. Additionally, the presence of brighter regions in the images suggested the incorporation of metallic species, further confirming the presence of Cu(II), Co(II), Ni(II) and Zn(II) ions in the synthesized molecules.

Energy-dispersive X-ray spectroscopy (EDX) mapping (Figs. 6 & 7) corroborated the elemental composition of the ligand and the Cu(II) complex. The ligand map (Fig. 6) displayed peaks corresponding to carbon (C), nitrogen (N), oxygen (O), and chlorine (Cl), consistent with the expected elements in its structure. Similarly, the Cu(II) complex map (Fig. 7) revealed the presence of these elements along with copper (Cu), confirming the successful incorporation of the metal ion into the complex. These findings, coupled with the diverse morphologies observed in SEM images strongly support the targeted synthesis of the complexes. Furthermore, the EDX data is consistent with the results of elemental analysis and theoretical calculations, providing further validation for the proposed structure of the complexes.

4.7. PXRD spectrum

X-ray diffraction (XRD) analysis was employed to characterize the morphology and crystallinity of the synthesized compounds (Figs. S12 and S13). The well-defined peaks in the XRD spectra indicated the crystalline nature of the samples [34]. The presence of new peaks

in the XRD patterns of the metal complexes compared to the ligand is a strong indicator of successful coordination between the metal ions and the ligand molecules. The emergence of these new peaks in the X-ray diffraction (XRD) pattern can be attributed to the formation of new crystallographic planes following complexation [35]. Furthermore, the Debye-Scherer formula was used to estimate the average crystallite size of the ligand and the Cu(II), Co(II), Ni(II), and Zn(II) complexes. The estimated sizes were 29, 32, 38, 17 and 52 nm, respectively.

5. Biological Studies

5.1. DNA interaction studies

5.1.1. Electronic absorption titration

Electronic absorption spectroscopy was employed to assess the binding interaction between the metal complexes with calf thymus DNA (CT-DNA) The spectra of both complexes displayed two strong absorption bands in the UV region. One band corresponds to ligand-to-metal charge transfer (LMCT), while the other arises from $\pi \rightarrow \pi^*$ transitions within the aromatic chromophores of the complexes [36]. The ability of a complex to intercalate between DNA base pairs depends on several factors, including the type of metal ion, chelation mode, ligand donor atoms, and the overall planarity of the complex. During intercalation, the aromatic chromophore of the complex stacks between DNA base pairs, typically leading to a hypochromic shift (decrease in absorbance) and a bathochromic shift (red shift) of the $\pi \rightarrow \pi^*$ transition band. The DNA binding interactions with Cu(II) and Zn(II) complexes are depicted in Figures S14 and S15, respectively.

Intercalating metal complexes typically exhibited hypochromism (decreased absorbance intensity) and a bathochromic shift (red shift) in their absorption spectra due to strong π-π^* stacking interactions between the complex's aromatic chromophore and DNA base pairs [37,38]. The Cu(II) complex displayed these characteristics, suggesting potential intercalative binding with CT-DNA. Upon increasing concentrations of CT-DNA, the absorption bands of all complexes exhibited hypochromism and a slight red shift of 4-7 nm, indicating a shift from blue to red wavelengths. These observations provided preliminary evidence for the intercalative binding mode of the Cu(II) complex with CT-DNA.

5.1.2. Viscosity gauges

Viscosity measurements offered a valuable tool to investigate changes in DNA length and elucidate the binding mode of metal complexes with DNA [39]. This technique is considered a reliable and sensitive method for assessing DNA binding interactions at room

temperature. For DNA binding studies, ethidium bromide (EB) served as a well-established intercalating agent. Upon intercalation between DNA base pairs, EB increased the viscosity of double-stranded DNA [40-44]. Similarly, changes in the ligand-to-metal charge transfer (LMCT) band upon the addition of DNA solution can provide insights into the binding mode of the synthesized complexes.

Changes in viscosity upon the addition of calf thymus DNA (CT-DNA) to the complexes (10^{-3} M) in a Tris-HCl/NaCl buffer (5 mM/50 mM, pH 7.2) were monitored (Fig. S16). Hypochromism, a decrease in absorption intensity, was observed, indicating potential intercalation of the complexes between DNA base pairs [45, 46]. This hypochromism is likely aroused from π-π^* stacking interactions between the aromatic chromophores of the complexes and the DNA bases. Notably, the ligand itself showed no significant binding, suggested that complex formation enhanced the intercalative ability. The intrinsic binding constant (K_b) values, calculated from the data in Fig. S16 and Table 2, quantify the strength of the complex-DNA interaction. Higher K_b values for the metal complexes compared to the ligand supported their superior binding affinity.

5.1.3. Antimicrobial screening

Antimicrobial function refers to the ability of a substance to kill or inhibit the growth of microorganisms like bacteria and fungi, thereby preventing or treating infections. In this study, the synthesized compounds were evaluated for their antibacterial and antifungal activity against selected bacterial and fungal strains using the microdilution method. Effective antimicrobial agents can act by disrupting the morphology (structure) and metabolism (cellular processes) of these pathogens, ultimately leading to their eradication or growth inhibition. The results of the antimicrobial assays are presented in Figs. 1 and 2 and enlisted in Tables 3, and 4.

The tables 3 and 4 compare the absorption properties of a ligand in its free form and when it is bound to different metal ions. They provided details about the shift in wavelength ($\Delta\lambda$) observed in the absorption spectra and the binding strength (K_b) for each complex. This data offered valuable insights into the interaction between the metal ion and the ligand, particularly how it affected the electronic properties of the resulting complex.

The minimum inhibitory concentration (MIC) values were determined to assess the potency of the synthesized compounds against various bacterial and fungal strains. A lower MIC value indicates a more potent compound, requiring a smaller dose to inhibit microbial growth. The microdilution broth method was employed to evaluate the in vitro antibacterial activity of the compounds against both Gram-positive (*Staphylococcus aureus, Bacillus*

subtilis) and Gram-negative (*Salmonella typhi, Escherichia coli, Klebsiella pneumoniae) bacteria, as well as against fungal strains (Aspergillus niger, Aspergillus flavus, Candida lunata, Candida albicans, Rhizopus bataticola*). Streptomycin and Nystatin served as standard controls for antibacterial and antifungal activity, respectively.

The observed enhanced activity of metal complexes compared to the free Schiff base ligand can be explained by Overton's principle and Tweedy's chelation theory [47,48]. Upon chelation with the metal ion, the positive charge is delocalized around the entire ring structure due to sharing with nitrogen and oxygen atoms. This reduces the overall polarity of the complex and increases its lipophilicity. As a result, the metal complex can more readily penetrate the lipid bilayer membrane of microbes. Additionally, these complexes may disrupt cellular respiration, hindering protein synthesis and ultimately limiting bacterial growth.

5.1.4. In vitro cytotoxicity assay

Chemotherapy leverages anti-tumor drugs to suppress the rapid, uncontrolled cell division characteristic of cancerous tumors. Promising results from prior DNA binding studies prompted an evaluation of the synthesized compounds' cytotoxic activity (ability to kill cancer cells) (Fig. 3). The MTT assay assessed the antiproliferative activity (inhibition of cell growth) against human cancer cell lines MCF-7 (breast) and Hep G2 (liver), along with a non-cancerous human breast epithelial cell line (HBL-100) as a control. Cisplatin served as a positive control for cell inhibition. The results, presented as IC_{50} values in Table 5, demonstrated that cell viability depends on the concentration of the compounds [49, 50]. Lower IC_{50} values indicated greater potency. As the concentration increased, the IC_{50} values decreased, revealing a dose and time-dependent cytotoxic effect.

Chelation therapy shows promise in targeting specific cancer cell types. The metal ions within these complexes plays a crucial role. During chelation, the positive charge of the metal can influence the ligand's ability to interact with protons, potentially affecting its overall effectiveness. Additionally, the planar structure of some chelates, facilitated by $\pi \rightarrow \pi^*$ interactions, might contribute to their cell-killing properties [51,52]. Interestingly, these complexes often exhibited reduced polarity due to charge stabilization, potentially enhancing their ability to penetrate cell membranes, as proposed by Tweedy's concept. This study suggested that copper complexes, compared to others, demonstrated superior anticancer activity against MCF-7 and Hep G2 cell lines. This improved efficacy could be attributed to a combination of factors, including the size and charge of the metal ion, steric effects of the ligand, and the complex's pharmacological properties.

5.1.5. Antioxidant activity

As antioxidants play a key role in combating free radicals, which are implicated in diseases like cancer and inflammation. this study revealed metal complexes to be superior antioxidants compared to their free ligand molecules. Although the ligand itself possessed some antioxidant activity, complex formation enhanced its ability to chelate metals and scavenge free radicals, offering better protection against various ailments. This improved efficiency is attributed to the ligand's structural features like conjugation, co-planarity, and the presence of heterocyclic atoms (details in Fig. 4 and Table 6).

6. Computational analysis

6.1. Predicting Biological Activity with PASS

Specifically, PASS online software was utilized to explore the biological activity of the Schiff base ligand (L), detailed in Table 7. The table suggested that the synthesized ligand may possess promising properties for various therapeutic applications, including potent anti-tuberculosis activity, antimycobacterial activity and antineoplastic activity (against cancers) - specifically for colorectal cancer, muscular dystrophy, colon cancer, and breast cancer, potent oxygen scavenger ability, kidney function stimulation, antiviral activity, mucositis treatment (treatment of inflammation of the mucous membranes).

It's important to remember that these predictions are based on insilico (computer-based) analysis and require further experimental validation to confirm their accuracy. However, the results from PASS provided valuable insights into the potential of the synthesized ligand as a drug candidate for various therapeutic targets. Specifically, PASS online software was utilized to explore the biological activity of the Schiff base ligand (L), detailed in Table 8. The table suggests that the synthesized ligand may possess promising properties for various therapeutic applications.

6.2. In-silico ADMET studies of ligand

In drug discovery, understanding a compound's Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties is critical. These properties influence how a drug interacts with the body and ultimately determine its suitability for further development. This study employed computational tools to predict the ADMET profile of a synthesized ligand, providing valuable insights into its potential as a drug candidate. SwissADME software was used to calculate various parameters crucial for understanding the drug's ADMET profile.

Log P: A Key Factor for Cell Permeability

Log P is a critical parameter used to assess a molecule's lipophilicity. In simpler terms, it reflects how much a molecule prefers an oily (lipophilic) environment compared to water (hydrophilic). This value played a significant role in determining how well a compound can pass through cell membranes. In drug discovery, particularly for medications targeting the central nervous system (CNS), Log P is a crucial indicator. The text highlighted that for ligands and complexes to effectively reach the CNS, their ideal Log P value (often denoted as Mi Log P) should be below 5. This suggested the compounds have enough lipophilicity to penetrate the fatty membrane barriers surrounding the CNS.

Molecular weight

The synthesized ligand's molecular weight (MW) of 332 falls within a range generally considered favorable for drug-like properties according to Lipinski's Rule of Five. This well-established rule serves. as a guideline in drug discovery to assess a molecule's potential for oral bioavailability. However, the molecular weights of the complexes formed by the ligand exceed 500. While this might suggest a decrease in drug-likeness compared to the free ligand, it's important to consider Lipinski's rule as a guiding principle rather than a strict exclusion criterion [53-55]. Many successful drugs have molecular weights exceeding this value.

Hydrogen bonding and protein interactions

This analysis explored the potential for hydrogen bonding in the compounds, a crucial factor for their interactions with protein binding pockets (active sites) where they exert their effects.

Schiff Base Ligand

The ligand displayed a well-balanced hydrogen bonding profile. It possesses 3 hydrogen bond acceptors and 1 hydrogen bond donor. This allows it to potentially form hydrogen bonds with both donor and acceptor groups on a target protein, potentially strengthening its interaction with the active site.

Metal Complexes

Interestingly, while the metal complexes cannot donate hydrogen bonds themselves (having 0 donors), they possess a higher number of hydrogen bond acceptors. This suggested they might rely primarily on accepting hydrogen bonds from surrounding molecules, potentially leading to different interactions with the active site compared to the free ligand.

Topological Polar Surface Area (TPSA) and Cell Permeability

TPSA is a valuable tool for estimating a molecule's exposed polar region, based primarily on the presence of hydrogen bond donors (like nitrogen and oxygen atoms with

attached hydrogens). This value, along with other factors, helps assess a compound's cell permeability, particularly its ability to passively diffuse through cell membranes.

For medications targeting the central nervous system (CNS), a lower TPSA is generally preferred. This allows for better passive diffusion across the blood-brain barrier (BBB), a selective gatekeeper that restricts the passage of molecules into the CNS. The study found that the ligand has a TPSA of 45.48 Å², while the complexes exhibited a higher TPSA of 94. This suggests the ligand might have superior passive permeability compared to the complexes, potentially making it more favorable for oral administration and delivery to the CNS.

Rotatable Bonds and Conformational Flexibility

Lipinski's rule of five also incorporated the concept of rotatable bonds within a molecule. These bonds allow the molecule to adopt various shapes, known as conformations. A certain level of flexibility can be advantageous for a drug candidate as it may enable it to better adapt to the specific conformation of the target protein's active site, potentially leading to stronger interactions.

This study revealed that the synthesized ligand possesses two rotatable bonds, while the metal complexes have eight. This suggested that both the ligand and the complexes might exhibit some degree of conformational flexibility, which could potentially be beneficial for their interactions with living cells.

Bioactivity Score and Drug Likeness

The bioactivity score of a molecule provided an indicator of its potential to interact with specific biological targets like proteins, enzymes, and receptors. These interactions are essential for a drug's pharmacological effect, or its impact on living organisms. The study found that both the metal chelates and the ligand have a bioactivity score of 0.

It's important to remember that bioactivity scores are often interpreted in conjunction with other factors, such as Lipinski's rule of five. This rule suggested that compounds with scores exceeding 0 generally exhibited better drug likeness properties. While the observed score of 0.55 falls below the proposed threshold for ideal drug-likeness according to Lipinski's rule, it doesn't necessarily exclude the compounds from further exploration. A comprehensive evaluation of their potential as drug candidates should consider other factors and experimental data.

The study utilized the SwissADME software (sometimes referred to as AdmetSAR) to perform an insilico (computer-based) toxicity assessment, specifically focusing on the potential for mutagenicity and carcinogenicity (cancer-causing properties) of the synthesized compounds. The evaluating potential toxicity early in drug development is crucial. The insilico

predictions from SwissADME suggested that the synthesized compounds may have a low risk of being mutagenic or carcinogenic. The results of the computational analysis on the drug-like properties of the synthesized compound are presented in Table 8.

6.3. **In silico Docking Studies**

6.3.1. *Docking simulations with protein*

Molecular docking simulations have emerged as a vital tool in computer-aided drug design by enabling the insilico prediction of ligand-receptor interactions [56]. Docking simulations were employed to evaluate the binding affinity of the synthesized ligands and their complexes, to the target protein, 3HB5. This technique facilitated rational drug design by simulating the interaction between small molecules (ligands) and macromolecules (receptors) like proteins [54]. Binding free energy, a key output of docking calculations, reflects the energy released upon ligand binding to the receptor. Lower binding free energy values indicated stronger and more favourable interactions. The 3HB5 structure serves as a springboard for exploring innovative therapeutic avenues. It inspires researchers to consider strategies beyond traditional medications. This could involve gene therapy or other techniques that target the enzyme itself or the estrogenic production pathway, opening doors to entirely new treatment options. The insilico docking simulations provided valuable insights into the interaction energies between the synthesized compounds and the target protein (3HB5). The calculated energies were -232.43 kJ/mol for the free ligand (L), and -264.14 kJ/mol, -263.07 kJ/mol, -261.92 kJ/mol, and -260.83 kJ/mol for the Cu(II), Co(II), Ni(II), and Zn(II) complexes, respectively. More negative interaction energies indicated stronger binding affinities [57]. These results suggested that the metal complexes form more favourable interactions with the target protein compared to the free ligand. Figs. 8 and 9 present the results of molecular docking simulations between DNA and the ligand, as well as the Cu(II) complex.

6.3.2. Docking simulations with DNA

Invitro binding studies were conducted to investigate the interaction between the synthesized compounds and DNA (PDB ID: 1BNA). The results suggested an intercalation binding mode, where the compounds inserted themselves between the base pairs of DNA. This interaction involves stacking interactions with the oxygen atoms of the DNA sugar-phosphate backbone, indicating that the entire molecule participates in binding to the targeted DNA macromolecule. The simulations revealed the ligand and its metal complexes positioned within the intercalative pocket of the target DNA, specifically within the base pair region. The calculated binding energy scores for the docked ligand-DNA complexes were favorable,

ranging from -205.25 kJ/mol (ligand) to -293.75 kJ/mol (Cu complex). The scores for the metal complexes with Co, Ni, and Zn were -283. 72, -271. 85 and -271.96 kJ/mol, respectively. These strong binding energies suggested potential stabilization by van der Waals forces and hydrophobic interactions. Figs. 10 and 11 show the results of molecular docking simulations for the ligand and the Cu(II) complex interacting with the protein.

Docking simulations provided valuable information on ligand-protein interactions. However, the data suggested that exploring alternative therapeutic strategies beyond traditional medications might be more fruitful for this target. Both in vitro assays and docking simulations consistently revealed an intercalative binding mode for the compounds with DNA. The entire ligand molecule participated in binding, likely through stacking interactions with the DNA backbone and further suggested stabilization by van der Waals and hydrophobic forces. The metal complexes exhibited strong binding affinities, with favorable binding energy scores. These results highlighted the potential of the metal complexes for further investigation. In conclusion, this study demonstrated the promising potential of the synthesized compounds for DNA targeting applications. Further research is necessary to explore their therapeutic efficacy and optimize their properties for drug development.

7. Conclusion

The fight against cancer continues to drive the development of new treatment options, particularly metal-based compounds. This research explored a novel approach using pyridine-based ternary complexes. Various spectral techniques proposed the octahedral structure of these metal complexes. The absorption spectra of all the complexes revealed an intense band around 320-340 nm, suggesting they interact with DNA through intercalation. This binding method often causes a bathochromic shift (19-28% increase in wavelength) and a hypochromic effect (decrease in intensity) in the absorption spectra. These effects likely arise from strong π-π^* stacking interactions between the aromatic rings in the complexes and the DNA base pairs. Molecular docking simulations corroborated these findings, providing additional support for the intercalative binding mode. This insilico technique strengthened the evidence for the compounds interacted with DNA.

The evaluation of the compounds' antibacterial and antifungal properties revealed the copper complex to be the most potent, with minimal inhibitory concentration (MIC) values ranging from 8 to 10 x 10^4 μM. Tweedy's chelation theory and the concept of overtones might explain this enhanced activity. The copper complex also displayed promising anticancer activity against MCF-7 and Hep G2 cell lines, with half maximal inhibitory concentration

(IC_{50}) values of 16 and 17 μM, respectively. Additionally, insilico ADMET predictions suggested the synthesized compounds exhibit favorable drug-like properties according to Lipinski's rule of five. These findings highlight the potential of these compounds, particularly the copper complex, as effective alternatives to platinum-based anticancer drugs. This research contributes significantly to the development of novel, potent, and potentially safer options for cancer treatment.

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Appendix A. Supplementary data

Supplementary data to this article was given separately.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest

References

1. M Ismael, A.M.M. Abdel-Mawgoud, M.K. Rabia, A. Abdou, Design and synthesis of three Fe (III) mixed-ligand complexes: Exploration of their biological and henoxazinone synthase-like activities, *Inorg. Chim. Acta*, 505 (2020) 119443-119948.
2. H. Sigel, R.B. Martin, Coordinating properties of the amide bond. Stability and structure on metal ion complexes of peptides and related ligands, *Chem. Rev.*,82(4) (1982) 385-426.
3. L. Li, Q. Guo, J. Dong, T. Xu, J. Li, DNA binding, DNA cleavage and BSAinteraction of a mixed-ligand copper (II) complex with taurine Schiff base and 1,10-phenanthroline. *J. Photochem. Photobiol. B: Biol.*, 125 (2013) 56-62.
4. C.X. Zhang, S.J. Lippard, New metal complexes as potential therapeutics, *Curr. Opin. Chem. Biol.*, 7(4) (2003) 481-489.
5. M.J. Reimann, D.R. Salmon, J.T. Horton, E.C. Gier, L. R. Jefferies, Water- soluble sulfonate Schiff-base ligands as fluorescent detectors for metal ions in drinking water and biological systems, *ACS omega*, 4(2) (2019) 2874-2882.
6. F.R. Keene, J.A. Smith, J.G. Collins, Metal complexes as structure-selective binding agents for nucleic acids, *Coord. Chem. Rev.,* 253 (15-16) (2009) 2021- 2035.
7. M. Komiyama, N. Takeda, H. Shigekawa, Hydrolysis of DNA and RNA by lanthanide ions: Mechanistic studies leading to new applications. *Chem. Comm.* 16, (1999) 1443-1451.
8. J. Zhou, M.R. Battig, Y. Wang, Aptamer-based molecular recognition for biosensor development, *Anal. Bioanal. Chem.*, 398 (2010) 2471-2480.
9. M.J. Hannon, J. Reedijk, Metal interactions with nucleic acids. *Dalton Trans.* 44(8) (2015) 3503-3504.
10. V. Brabec, J. Kasparkova, Ruthenium coordination compounds of biological and biomedical significance. DNA binding agents, *Coord. Chem. Rev.*, 376 (2018) 75-94.
11. Y. M. Song, Q. Wu, P.J. Yang, N.N. Luan, L.F. Wang, Y. M. Liu, DNA binding and cleavage activity of Ni(II) complex with all-trans retinoic acid, *J. Inorg. Biochem.*, 100 (10) (2006) 1685-1691.
12. A.M. Beatty, Open-framework coordination complexes from hydrogen bonded networks: toward host/guest complexes, *Coord. Chem. Rev.*, 246(2) (2003) 131-143.
13. M. Zhao, H.B. Wang, L.N. Ji, Z.W. Mao, Insights into metalloenzyme microenvironments: biomimetic metal complexes with a functional second coordination

sphere, *Chem. Soc. Rev.*, 42(21) (2013) 8360-8375.

14. G. Mohammad Abu-Taweel, M.M. Ibrahim, S. Khan, H.M. Al-Saidi, F.A Alshamrani, M., Alhumaydhi, S.S. Alharthi,, Medicinal importanceand chemosensing applications of pyridine derivatives: A review, *Crit. Rev. Anal. Chem.*, 54(3) (2024) 599-616.
15. F. Borges F. Roleira, N. Milhazes, L. Santana, E. Uriarte, Simple coumarins and analogues in medicinal chemistry: occurrence, synthesis and biological activity, *Curr. Med. Chem.*, 12(8) (2005) 887-916.
16. R. Ranjith, The chemistry and biological significance of imidazole, benzimidazole, benzoxazole, tetrazole and quinazolinone nucleus, *J. Chem. Pharm. Res.*, 8(5) (2016). 505-526.
17. S. De, A.K. SK, S.K. Shah, S. Kazi, N. Sarkar, S. Banerjee, S. Dey, Pyridine: The scaffolds with significant clinical diversity, *Rsc Adv.*, 12(24) (2022) 15385- 15406.
18. A. Chiacchio, M. Daniela Iannazzo, R. Romeo, V. Salvatore Giofre, L. Legnani, Pyridine and pyrimidine derivatives as privileged scaffolds in biologically active agents, *Curr. Med. Chem.* 26, 40 (2019) 7166-7195.
19. A.Z. El-Sonbati, N.F. Omar, M.I. Abou-Dobara, M.A. Diab, M.A. El-Mogazy, Sh M. Morgan, M.A. Hussien, A. A. El-Ghettany, Structural, molecular docking computational studies and in-vitro evidence for antibacterial activity of mixed ligand complexes, *J. Mol. Struct.* 1239 (2021) 130481-130489.
20. A.H. Valekar, M. Lee, J.W. Yoon, J. Kwak, D.Y., K.R. Hong, Oh, Y.K. Hwang, Catalytic transfer hydrogenation of furfural to furfuryl alcohol under mild conditions over Zr-MOFs: Exploring the role of metal node coordination and modification, *ACS Catal.*, 10(6) (2020) 3720-3732.
21. A. Singh, H.P. Gogoi, P. Barman, Comparative study of palladium (II) complexes bearing tridentate ONS and NNS Schiff base ligands: Synthesis, characterization, DFT calculation, DNA binding, bioactivities, catalytic activity, and molecular docking. *Polyhedron*, 221 (2022) 115895.
22. G.C. Xu, L. Zhang, L. Liu, G.F. Liu, D.Z. Jia, Syntheses, characterization and crystal structures of mixed-ligand Cu(II), Ni(II) and Mn(II) complexes of N-(1-phenyl-3-methyl-4-propenylidene-5-pyrazolone)-salicylidene hydrazide containing ethanol or pyridine as a co-ligand, *Polyhedron*, 27(1) (2008) 12-24.

23. R.L. Alanazi, M. Zaki, W.A. Bawazir, Synthesis and characterization of the new ligand, 1, 2, 4-triazino [5,6-b] indol-3-ylimino methyl naphthalene-2-ol and its Ni(II) and Cu(II) complexes: Comparative studies of their in vitro DNA and HSA Binding, *BioMetals*, 35(6) (2022) 1199-1223.
24. M. Mishra, K. Tiwari, S. Shukla, R. Mishra, V.P. Singh, Synthesis, structural investigation, DNA and protein binding study of some 3d-metal complexes with N′-(phenyl-pyridin-2-yl-methylene)-thiophene-2-carboxylic acid hydrazide, *Spectrochim. Acta Part A: Mol. Biomol. Spect.*, 132(2014) 452-464.
25. K.M. Ibrahim, I.M. Gabr, R.R. Zaky, Synthesis and magnetic, spectral and thermal eukaryotic DNA studies of some 2-acetylpyridine-[N-(3-hydroxy-2-naphthoyl)] hydrazone complexes, *J. Coord. Chem.*, 62(7) (2009) 1100-1111.
26. P. Anitha, R. Manikandan, P. Vijayan, G. Prakash, P. Viswanathamurthi, R.J. Butcher, Nickel (II) complexes containing ONS donor ligands: Synthesis, characterization, crystal structure and catalytic application towards CC cross-coupling reactions, *J. Chem. Sci.*, 127 (2015) 597-608.
27. P.B Sreeja., M.P. Kurup, A. Kishore, C. Jasmin, Spectral characterization, X-ray structure and biological investigations of copper (II) ternary complexes of 2-hydroxyacetophenone 4-hydroxybenzoic acid hydrazone and heterocyclic bases. *Polyhedron*, 23(4) (2004) 575-581.
28. A. Prakash, R. Malhotra, Co(II), Ni(II), Cu(II) and Zn (II) complexes of aminothiazole-derived Schiff base ligands: Synthesis, characterization, antibacterial and cytotoxicity evaluation, bovine serum albumin binding and density functional theory studies, *Appl. Organomet. Chem.*, 32(2) (2018) 4098.
29. S. Savir, J.W.K. Liew, I. Vythilingam, Y.A.L. Lim, C.H. Tan, K.S. Sim, K.W Tan, Nickel (II) complexes with polyhydroxybenzaldehyde and O, N, S tridentate thiosemicarbazone ligands: Synthesis, cytotoxicity, antimalarial activity, and molecular docking studies, *J. Mol. Struct.*, 1242 (2021) 130815.
30. W. Bal, M. Sokołowska, E. Kurowska, P. Faller, Binding of transition metal ions to albumin: sites, affinities and rates, *Biochim. Biophys. Acta, Gen. Subj.,* 1830(12) (2013) 5444-5455.
31. T.A. Yousef, G.A. El-Reash, O.A. El-Gammal, R.A. Bedier, Co(II), Cu(II), Cd(II), Fe(III) and U(VI) complexes containing a NSNO donor ligand: Synthesis, characterization, optical band gap, in vitro antimicrobial and DNA cleavage studies, *J.*

Mol. Struct., 1029 (2012) 149-160.

32. V.K. Gupta, A.N. Bhat, Synthesis and characterization of anti-2-furan carboxaldoxime complexes with Cobalt (II), Copper (II & I) and Silver (I), *J. Coord. Chem.*, *8*(4) (1979) 183-189.
33. S.M. Sheta, M.A. H.E. Akl, Saad, E.S.R. El-Gharkawy, A novel cerium (III)–isatin Schiff base complex: spectrofluorometric and DFT studies and application as a kidney biomarker for ultrasensitive detection of human creatinine, *RSC Adv.*, 10(10) (2020) 5853-5863.
34. T. Bharanidharan, A. Senthil, Crystal growth, spectral, Hirshfeld surface analysis, and physicochemical properties of third-order non-linear optical single crystal: 2-methylimidazolium hydrogen adipate, *J. Mol. Struct.*, (2023). 1272: 134145.
35. L.H. Abdel-Rahman, A.M. Abu-Dief, R.M. El-Khatib, S.M. Abdel-Fatah, Some new nano-sized Fe (II), Cd (II) and Zn (II) Schiff base complexes as precursor for metal oxides: Sonochemical synthesis, characterization, DNA interaction, in vitro antimicrobial and anticancer activities. *Bioorg. Chem.*, 69 (2016)140-152.
36. K.F. Chung, P. Godard, E. Adelroth, J. Ayres, N. Barnes, P. Barnes, P. Sterk, Difficult/therapy-resistant asthma: the need for an integrated approach to define clinical phenotypes, evaluate risk factors, understand pathophysiology and find novel therapies. ERS Task Force on Difficult/Therapy-Resistant Asthma. European Respiratory Society. *Eur. Respir. J.* 13(5) (1999) 1198-1208.
37. P.J. Cox, G. Psomas, C.A. Bolos, Characterization and DNA-interaction studies of 1, 1-dicyano-2, 2-ethylene dithiolate Ni (II) mixed-ligand complexes with 2- amino-5-methyl thiazole, 2-amino-2-thiazoline and imidazole. Crystal structure of [Ni (i-MNT) (2a-5mt) 2]. *Bioorg. Med. Chem.* 17(16) (2009) 6054-6062.
38. J. Liu, T. Zhang, T. Lu, L. Qu, H. Zhou, Q. Zhang, L. Ji, DNA-binding and cleavage studies of macrocyclic copper (II) complexes, *J. inorg. Biochem.*, 91(1) (2002) 269-276.
39. F. Arjmand, M. Muddassir, Design and synthesis of heterobimetallic topoisomerase I and II inhibitor complexes: In vitro DNA binding, interaction with 5′- GMP and 5′-TMP and cleavage studies, *J. Photochem. Photobiol. B: Biol.*, 101(1) (2010) 37-46.
40. S. Rudra, S. Dasmandal, C. Patra, A. Kundu, A. Mahapatra, Binding affinities of Schiff base Fe (II) complex with BSA and calf-thymus DNA: Spectroscopic investigations and molecular docking analysis. *Spectrochim. Acta Part A: Mol. Biomol. Spect.*, (2016) 166 84-94.
41. C. Nicolini, N. Claudio, Normal Cells and Cancer Cells: Macromolecular Structures

and Cellular Functions, *Biophysics and Cancer* (1986): 1-135.

42. G.T. Vidyavathi, B.V. Kumar, A.V. Raghu, T. Aravinda, U. Hani, H.A Murthy, A.H. Shridhar, Punica granatum pericarp extract catalyzed green chemistry approach for synthesizing novel ligand and its metal (II) complexes: Molecular docking/DNA interactions, *J. Mol. Struct.*, (2022) 1249 131656.
43. M.Z. Iqbal, A.U. Rehman, S. Siddique, Prospects and challenges of graphene based fuel cells. *J. Energy Chem.*, 39 (2019) 217-234.
44. N.N. Muzammil, M.T.M. Ali, A.M. Tajuddin, Synthesis and characterization of palladium(ii) Schiff base complexes derived from phenylamines and their catalytic activity for stilled reaction, *M.J. Anal. Sci.,* 28(2) (2024) 335-347.
45. N. I. Shtemenko, H.T. Chifotides, K.V. Domasevitch, A.A. Golichenko, S.A. Babiy, Z. Li, K.R. Dunbar, Synthesis, X-ray structure, interactions with DNA, remarkable in vivo tumor growth suppression and nephroprotective activity of cis- tetrachloro-dipivalato dirhenium (III), *J. Inorg. Biochem.*, 129 (2013) 127-134.
46. A. Kellett, Z. Molphy, C. Slator, V. McKee, N.P. Farrell, Molecular methods for assessment of non-covalent metallodrug–DNA interactions, *Chem. Soc. Rev.*, 48(4) (2019) 971-988.
47. H. Wariishi, K. Valli, M.H. Gold Manganese (II) oxidation by manganese peroxidase from the basidiomycete Phanerochaete chrysosporium. Kinetic mechanism and role of chelators, *J. Biol. Chem.*, 267(33) (1992) 23688-23695.
48. A. A. El-Sherif, T.M. Eldebss, Synthesis, spectral characterization, solution equilibria, in vitro antibacterial and cytotoxic activities of Cu (II), Ni (II), Mn (II), Co (II) and Zn (II) complexes with Schiff base derived from 5-bromosalicylaldehyde and 2-aminomethylthiophene, *Spectrochim. Acta Part A: Mol. Biomol. Spect.*, 79(5) (2011) 1803-1814.
49. J.E. Talmadge, R.K. Singh, I.J. Fidler, A. Raz, Murine models to evaluate novel and conventional therapeutic strategies for cancer, *Am. J. Pathol.*, 170(3) (2007) 793-804.
50. S. Parveen J.A. Cowan, Z. Yu F. Arjmand, Enantiomeric copper based anticancer agents promoting sequence-selective cleavage of G-quadruplex telomeric DNA and non-random cleavage of plasmid DNA, Metallomics (2020) 12(6)988-999.
51. H. Sigel, R.B. Martin, Coordinating properties of the amide bond, Stability and structure of metal ion complexes of peptides and related ligands, *Chem. Rev.,* 82(4) (1982) 385-426.
52. F.P. Bossu, D.W. Margerum, The stabilization of trivalent nickel in deprotonated-

peptide complexes, *J. Am. Chem. Soc.*, 98 (13) (1976) 4003-4004.

53. C. Agoni, F.A. Olotu, P. Ramharack, M.E. Soliman, Druggability and drug-likeness concepts in drug design: are biomodelling and predictive tools having their say? *J. Mol. Model.*, 26 (2020) 1-11.
54. T.H. Keller, A. Pichota, Z. Yin, A practical view of 'druggability'. *Curr. Opin. Chem. Biol.*, 10(4) (2006) 357-361.
55. B.G. Ugarkar, J.M DaRe, J. J. Kopcho, C.E., Browne, J.M. Schanzer, J.B. Wiesner, M. D. Erion, Adenosine kinase inhibitors. 1. Synthesis, enzyme inhibition, and antiseizure activity of 5-iodotubercidin analogues, *J. Med. Chem.,* 43(15) (2000) 2883-2893.
56. A.A. Naqvi, T. Mohammad, G.M. Hasan, M.I. Hassan, Advancements in docking and molecular dynamics simulations towards ligand-receptor interactions and structure-function relationships, *Curr. Top. Med. Chem.*, 18(20) (2018) 1755-1768.
57. S. Henrich, I. Feierberg, T. Wang N. Blomberg R.C. Wade, Comparative binding energy analysis for binding affinity and target selectivity prediction, Proteins: Struct., Funct., Bioinf. 2010 Jan;78(1):135-53.