RESEARCH ARTICAL

Synthesis, DNA Binding, Molecular Docking and Anticancer Studies of Copper (II), Nickel (II), and Zinc (II) Complexes of Primaquine-based Ligand

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ABSTRACT

In a research study, a metal-based complex demonstrated anticancer properties. The process involved the synthesis of a compound called 7-chloro-N-[5-(diethylamino)pentan-2-yl]-N-[sulfanyl(carbonothioyl)]quinolyn-4-amine, which incorporated a primaquine-based dithiocarbamate ligand. This synthesis method utilized sodium hydroxide and carbon disulfide at temperatures of 0 to 5°C. Nickel(II), copper(II), and zinc(II) ions were employed as the metal-complexed ligands through various chromatographic and spectroscopic techniques that enhanced their purity. Metal compounds, such as cisplatin and its analogs, were widely used to treat different cancer types. Presently, researchers are exploring alternative metal-based complexes as potential agents against cancer, aiming to mitigate toxic effects linked to medications made of platinum. Investigations conducted *in-silico* revealed that the ligand preferred interacting with DNA's minor groove, assembling a single hydrogen bond between an adenine hydrogen atom and the oxygen element of the carbonyl compound in the pyrrolidinone unit. Compared to cisplatin, copper complexes can overcome drug resistance by lowering their toxicity. The findings indicate that these primaquine analogs have the potential to lay the foundation for a novel and efficacious category of cancer chemotherapeutics.

Keywords: Metal complexes, Copper ligand, DNA interaction, Molecular docking

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INTRODUCTION

Cancer has become a significant global threatful disease, with modernization playing a major role in its increasing incidence.[1] Despite the availability of numerous medications for its treatment, millions of lives are lost to cancer every year. Additionally, the usage of current anticancer medications frequently results in serious adverse effects for survivors. Metal-based drugs like cisplatin and its next-generation counterpart, carboplatin, were used in the therapy of some tumors for a long time. Unfortunately, these medications have a number of adverse effects, such as ototoxicity, neurotoxicity, and renal impairment.[2] The accumulation of these facts urged researchers to explore the evolution of innovative potential anticancer medicines based on non-platinum metallic complexes. Copper, nickel, and zinc, all dithiocarbamate complexes with various ions made up of transition metals have emerged as the top candidates for such endeavors. Metal dithiocarbamates[3-5] have shown significant inhibitory effects on NF-kappa B, the pivotal player, a key component of many human malignancies and in the body's immune response. Primaquine, a medication with a longstanding history in medicine, falls under the category of antimalarials. Beyond its established role in malaria treatment, primaquine has met attention for its potential in addressing diverse medical

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conditions. It has been investigated as a potential treatment for autoimmune disorders like rheumatoid arthritis and lupus due to its immunomodulatory effects. Additionally, interest has been paid to its antiviral properties, particularly in combating viruses such as human immunodeficiency virus (HIV) and specific types of coronaviruses. Primaquine continues to grasp significance in the battle against malaria and has contributed to the exploration of treatments for various other diseases. Its evolution from being recognized primarily for its antimalarial properties to finding potential applications in a wide range of medical contexts underscores its enduring impact on global health. Designing copper, nickel, and zinc compounds as potential treatments for cancer has garnered a lot of interest. Specific organocopper compounds are thought to possess anticancer properties due to their capacity to scavenge superoxide, which is demonstrated by the activity of complexes composed of copper with low molecular mass that are similar to cu-zinc-superoxide dismutase. Notably, Ali *et al*. recently released information on copper (II), nickel (II), and zinc (II) complex produced from a glutamic acid-based ligand and their DNA bonding and cancer prevention properties.[6] Their research revealed effective DNA binding and strong antiproliferative properties contrary to the human cancer cell lines HT-29, MDA-MB-231, HepG2, and HeLa. Due to nickel's connection to DNA and specific proteins that bind to DNA, there aren't many studies investigating the cancer-fighting abilities of nickel combinations in the existing research. Copper complexes were the topic of intense research over the past years, with two of them successfully making it to clinical trials. With these findings in mind, a dithiocarbamate ligand (L) based on primaquine was created by reacting primaquine with carbon disulfide under the evidence of sodium hydroxide. Ni (II), Cu (II) and Zn (II) ions were used to complex the resultant ligand in various ways.[7,8] Purification and characterization of the ligand and its complexes were performed utilizing a variety of chromatographic and spectroscopic methods. Utilizing ultraviolet spectrophotometry, their stabilities at physiologic pH values have been evaluated. Additionally, the ligand was subjected to *in-silico* studies to determine the method of DNA binding. Spectrophotometric techniques were used to carry out binding of DNA experiments for the ligand and its various complexes, and the binding parameters were then determined.

MATERIALS AND METHODS

Materials and Techniques

Analytical reagents (AR) level reagents were all purchased. A Gujarat-based supplier for Indexim International provided the primaquine phosphate. The supplier of the carbon disulfide was SD Fine Chemicals, Ltd. in Mumbai, a city in India. The following substances have been bought: $NiCl_{26}H_2O$, $CuCl_{22}H_2O$, $ZnCl_{22}H_2O$, ethanol, methanol, DMF and hexane. From E. Merck in Germany, coated aluminum silica gel 60 F254 lightweight plates had already been bought. Tris-(hydroxymethyl) aminomethane and the disodium salt of Ct-DNA originated from sisco The research Lab in Mumbai, an Indian city.[24-25] The electrospray ionizing radiation mass spectrum (ESI-MS) was conducted out in the negative mode with a ZQ-4000 single quadrupole mass spectrometer. Using a Bruker Advance 500 and a the Varian Unity + 300 spectrometer, nuclear magnetic resonance (NMR) spectra were documented in DMSO. Chemical changes are expressed in parts per million (ppm) in proportion to tetramethyl silane. Employ a CHN 2400 analyzer from Perkin Elmer.

Ligand Synthesis (l)

Housing a solution of thalidomide (832.22 mg; 3.1 mM) in methan-1-ol (20mL), a round-bottom flask of 250 mL was filled with carbon disulfide (232.63 mL; 3.1 mM). This reaction was carried out at a temperature ranging from 0 to 5°C in a bath of ice water. The mixture was agitated consistently for a duration of 4 hours.[9-11] An analogous sodium hydroxide solution of up to 2 mL was incorporated into the reaction compound. For an additional 8 hours, the ultimate mixture compound was continuously stirred until it assumed a fully yellow hue. TLC confirmed the end of the reaction . The yellowish ligand compound was then diminished by two-thirds of its original volume, followed by storage in a refrigerator to precipitate the complex ligand. An amorphous polydentate, that is, the resultant yellow solid, was subjected to a wash with hexane, further retrieved from methanol; ultimately, it was dehydrated in a desiccator under vacuum conditions that included fused calcium chloride.

Synthesis of Complexes

A solution containing 41.02 mg (0.2 mM) of copper chloride dihydrate in methan-1-ol (10 mL) was introduced into an agitated mixture of the ligand [12-15] weighing 75.8 mg (0.2 mM), dissolved in methan-1-ol (15 mL). The resultant compound was agitated at ambient conditions until a complex settled solution was generated. The precipitate that was produced was filtered along with subsequent rinsing with hexane and chilled methanol.[16-21] Nickel and zinc compounds were formulated using a similar method. Ultimately, it was dehydrated in a desiccator under vacuum conditions, including fused calcium chloride.

Solution Stability

PBS solution (with 5% DMSO) helped observe their UV–vis spectrum for 24 hours to acquire an appropriate comprehension of the compound stabilization in a mixture under physiological pH conditions.[22] NiL, CuL, and ZnL were initially liquefied in a minimal quantified DMSO, which was further adulterated in PBS at a pH of 7.4 to achieve a 10^{-4} M concentrated solution.[23-26] The compounds were assayed on the basis of their hydrolysis patterns by recording the ligand spectra over a 24-hour time period at a constant temperature of 25ºC.

DNA Binding

UV- vis spectrophotometry was utilized to explore the reaction between the compound and its ligand complex with Ct-DNA. 2-amino-2-(hydroxymethyl)-1,3-propanediol , THAM (Tris, 10-2 M, pH 7.4) was solubilized with sterilized distilled water for

the experiment. With an annihilation value of 6600 M^{-1} cm^{-1} at 260 nm, spectrophotometry was utilized to establish the molar volume of the disodium salt solution of Ct-DNA.[27,28]Gradually, higher concentrations of DNA was added to conduct the binding experiments (ranging from 9.3×10^{-5} to 9.9×10^{-5} M), counteracting a constant quantity of the metal complexes and the ligand (1.6×10^{-4} M). The maximum absorption (k_{max}) and absorbance in buffer solutions of metal complexes and pure DNA were initially documented. Subsequently, each DNA solution (2 mL) and either the ligand or metal complex solution were combined, and their respective k_{max} along with its absorbance assays were deduced. About 2 mL of DNA solution at each step were recorded following the addition of varying concentrations of the absorption.

In-silico Studies

The docking studies involved several steps. Firstly, AutoDock Vina was utilized to perform the docking, with the DNA structure recorded in PDB format while excluding heteroatoms.[22-26] Next, Gastegier charges were allocated to the DNA and saved in PDBQT form *via* AutoDock tools (ADT). The complexes in DMSO were evaluated for molar conductance in atmospheric temperature.[28-32] The complexes in DMSO were measured for its conductance at room temperature. Marwin Sketch was utilized to generate the 3D structure of the ligand, which was subsequently converted to a pdb file extension. Gastegier charges were assigned for ligand synthesis, non-polar hydrogens were combined, and it was saved in PDBQT form again *via* ADT. The protein data bank is where X-ray crystallized form of DNA (PDB ID: 6LUD) was identified. Again, the DNA was converted to a PDB format using ADT, omitting heteroatoms. With AutoDock Tools (ADT), Gastegier charges were allocated, non-polar hydrogens were merged, and it was once more preserved in PDBQT format. The Protein Data Bank is where the X-ray crystallized form of DNA (PDB ID: 6LUD) was found. ADT was once more used to assign Gastegier charges to the DNA and record it in PDBQT format. Grid and docking files were determined *via* ADT. With AutoDock 4.0 (Scripps Research Institute, USA), the actual docking study was carried out while treating the rotatable bonds of the ligand as flexible and the receptor as stiff. The finished DNA structure was enclosed by a grid of size 60 by 80 by 114 and a spacing of 0.375, excluding heteroatoms. Lamarckian Genetic Algorithm and free energy function were utilized in the docking of the macromolecule, with a population of 150 arbitrarily chosen individuals, and an utmost occupant of 2500000 energy analysis, a variation rate of 0.02, and an overlap rate of 0.80. Every ligand and DNA-ligand complex underwent fifty independent docking trials in order to identify the least free energy binding conformation. These runs were recorded in PDBQT format. Finally, UCSF Chimera was used to analyze the docking results for potential hydrophilic and hydrophobic interactions.

RESULTS AND DISCUSSION

Analytical and spectroscopic data provided the proposed structures of copper, nickel, and zinc compounds along with their respective ligands. All compounds existed as non-crystalline solids. The ligand showed slight sensitivity to air, while the complexes were notably stable. All of them could be easily dissolved in methanol, DMSO, and DMF. CuL, NiL, and ZnL yields were 83.6, 72.0, and 79.0%, respectively. The chemicals were extracted from methanol after being purified in cold methanol and hexane washes. In the presence of sodium hydroxide, primaquine carried out a process of substitution with carbon disulfide, replacing a hydrogen atom on the 2º amide N of the ring. Figure 1. Represented the 2º amide N of primaquine attacking the carbon of CS_2.

This took place in a chilly system (between 0 and 5 °C). The resultant dithiocarbamate L bound with the ions of copper (II), nickel (II), and zinc (II) to produce CuL, NiL, and ZnL. (Show in Figure 2). Copper and zinc complexes with low molar conductance values were likely to incorporate two chloride ligands in the coordination sphere. CuL, on the other hand, had conductance like that of a 1:2 electrolyte, suggesting the presence of two chloride anions beyond the complex's coordination sphere. It was concluded that bimetallic complexes were produced based on the results of the elementary evaluation and ESI-MS spectra.CuL, NiL, and ZnL all possessed 3 isomeric forms: (R, R-), (S, S-), & (R, S-), whereas the ligand had 2 isomeric forms (R- and S-). Figure 3 displays various potential stereoisomeric configurations of the L and its Cu complex. In 5% DMSO solutions of phosphate buffer solutions at a pH of 7.4, all the complexes showed stability, proving that the chloride ligands were resistant of being displaced by solvent molecules.

The nickel, zinc, and copper complexes had an octahedral environment, while the copper complexes had a tetragonal environment that were corroborated by their respective UV spectra.

Ligands

Copper ligand

^{1}H-NMR (500MHz,MeOD)d: 8.54 (1H;d;J = 4.1 Hz;H2); 8.23 (1H;d;J = 7.1 Hz;H5); 7.92 (1H;d;J = 1.1Hz;H8); 7.37 (1H;dd;J = 7.12 and 1.2 Hz;H6); 6.96 (1H;d;J = 3.4 Hz;H3); 5.92 (2H;t;J =

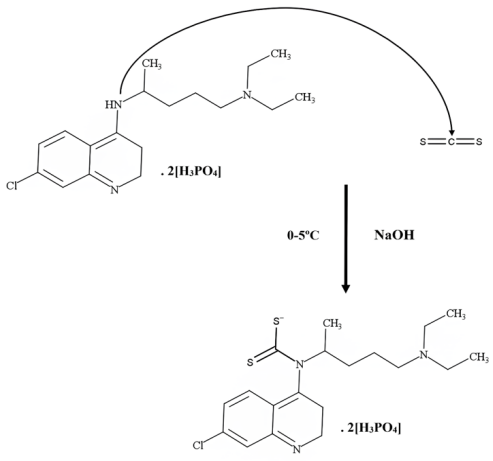

Figure 1: A diagram illustrating the 2º amide N of primaquine attacking the carbon of CS2

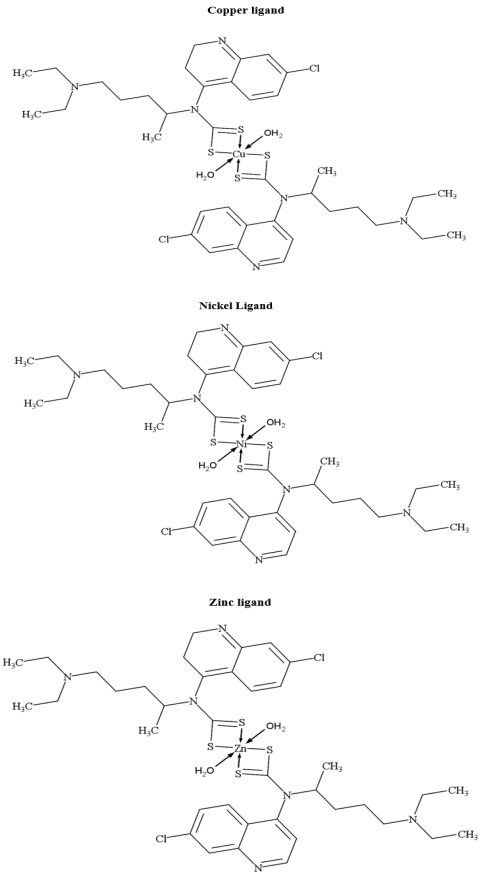

Figure 2: Synthesis of primaquine dithiocarbamate (L) and its Cu, Ni and Zn complexes.

4.54 Hz;H10 or H20); 3.73 (2H;t;J = 4.0 Hz;H10 or H20); 2.64 (2H;m;H10 0); 2.63 (2H;m;H20 0); 2.48 (2H;m;H30 0); 2.20 (3H;q;J = 4.2 Hz;H40)ppm; NMR-13C (125MHz,MeOD)d:151 .40;149.20;141.46;133.20;127.40;126.81;125.66;124.30;123.60;1 11.20;55.30;52.10;46.50;32.91;22.50;18.01;11.44 ppm.

IR mmax (cm_1;KBr pellets):3233 (N-H);1545 (C = C).

MS/ESI:m/z[M H]+:887.64

Molecular weight: 889.58

Isotope formula: $C_{38}H_{54}C_{l2}CuN_6O_2S_4$

Nickel ligand

^{1}H-NMR (500MHz,MeOD)d:8.54 (1H;d;J = 4.1Hz;H2);8.23 (1H;d;J = 7.1Hz;H5);7.92 (1H;d;J = 1.1 Hz ;H8) ;7.37 (1H;dd;J = 7.12 and 1.2 Hz;H6); 6.96 (1H;d;J = 3.4 Hz;H3); 5.92 (2H;t;J = 4.54 Hz;H10 or H20);3.73 (2H;t;J = 4.0 Hz;H10 or H20);2.64 (2H;m;H100);2.63 (2H;m;H200);2.48 (2H;m;H300) ;2.20 (3H;q;J = 4.2;H40)ppm;NMR-13C (125MHz,MeOD)d:151.40 ;149.20;141.46;133.20;127.40 ;126.81;125.66;124.30;123.60;111 .20;55.30;52.10;46.50;32.91;22.50;18.01;11.44 ppm.

IR mmax (cm^{-1};KBr pellets):3233 (N-H);1545 (C = C).

MS/ESI:m/z[M H]+: 884

Molecular weight: 884.72

Isotope formula: $C_{38}H_{54}C_{l2}N_6NiO_2S_4$

Zinc ligand

^{1}H-NMR (500MHz,MeOD)d:2.64 (1H;d;J = 4.1Hz;H2);2.63 (1H;d;J = 7.1Hz;H5);2.59 (1H;d;J = 1. 1Hz;H8); 2.54 (1H;dd;J = 7.12 and 1.2 Hz;H6); 2.48 (1H;d;J = 3.4 Hz;H3); 2.20 (2H;t;J = 4.54 Hz;H10 or H20);1.97 (2H;t;J = 4.0 Hz;H10 or H20);1.92 (2H;m;H100);1.74 (2H;m;H200);1.50 (2H;m;H300) ;1.47 (3H;q;J = 4.2 Hz;H40)ppm; NMR-13C (125MHz,MeOD) d:123.60;111.20; 55.30;53.01;52. 10;49.24;46.68;46.50;33.42;3 2.91;26.03;22.50;19.26;18.01;11.44;11.08 ppm.

IR mmax (cm_1;KBr pellets):3233 (N-H);1545 (C = C).

MS/ESI:m/z[M H]+:891

Molecular weight: 891.41

Isotope formula: $C_{38}H_{54}C_{l2}N_6O_2S_4Zn$

Molar Conductance Measurements

In DMSO, the compounds were measured for molar conductivity at room temperature. Molar conductance measurements on the complexes in DMSO were performed when they were at room temperature on the DMSO complexes. Copper, nickel, and zinc complexes in 10-3 M solutions had corresponding molar conductances of 123.31, 111.12, and 118.31 cm^2 mol^{-1}. These findings showed that the ruthenium complex had a 1:2 electrolytic nature while the copper and zinc complexes did not. As a result, it may be inferred that the chloride ions in CuL and ZnL successfully balanced the electrical charge and ligancy of the Cu^{2+} and Zn^{2+} ions within their complexes, so demonstrating their presence within the coordination spheres. Therefore, it is plausible that NiL and ZnL have tetragonal and octahedral geometries. Two chloride ions are positioned on the external environment to counteract the extra positive charge from the two Cu^{3+} ions in the case of CuL.

Elemental Analysis

The constituents of L, CuL, NiL, and ZnL were identified through elementary analysis. The calculated and recorded proportions of H, C, N and S were very similar. The potential molecular formulas for L, CuL, NiL, and ZnL can be represented as follows using this information on elemental composition and the results of molar conductance studies.

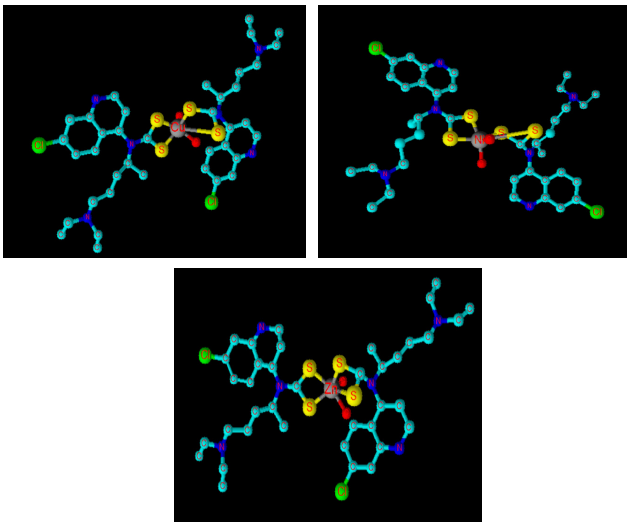

Figure 3: Stereoisomers of L and CuL,NiL and ZnL obtained *via* Marvin Sketch 5.8.2.

Table 1: Molecular Docking Analysis of Synthesized Compounds with DNA interaction

Sl No	*Compound*	*Docking score*
1	Copper-Ligand	-7.8
2	Nickel-Ligand	-6.5
3	Zinc-Ligand	-7.2
4	Ligand	-6
5	Cisplatin(std)	-8.5

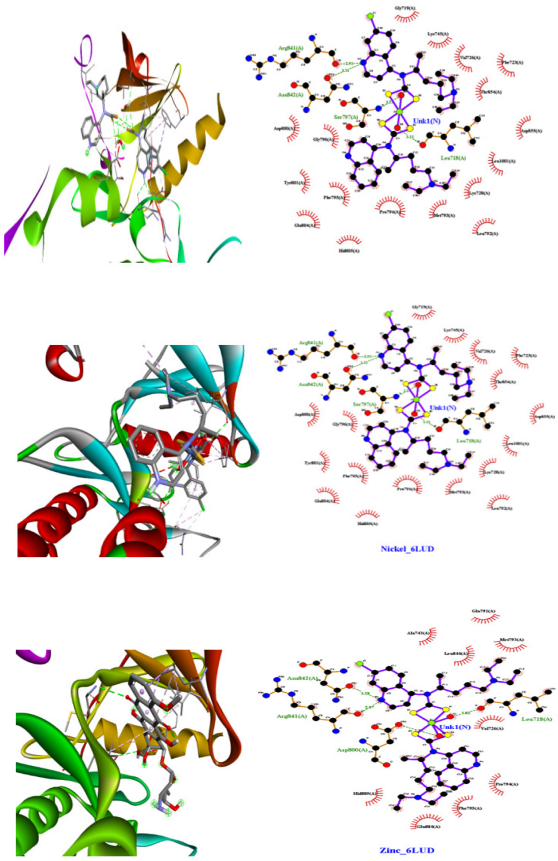

Figure 4: Molecular docking model demonstrating the interaction of ligand with the DNA Ligplot of Cul, NiL and ZnL

Molecular Docking

The L, CuL, NiL, and ZnL were predicted to have DNA binding constants (Kb) of 3.4 x 104, 1.3 x 105, 3.2 x 104, and 3.8 x 104 M^{-1}, respectively. The calculated values strongly imply a substantial affinity for DNA binding for both the ligand and its complexes (Show in Table 1). When compared to their unbound ligands, metal complexes showed a noticeably better affinity for DNA. The existence of a second electrical charge on the metal ion core and unoccupied d-orbitals in the complexes are thought to be the causes of this improved binding. It is notable that L obtained the lowest DNA binding constant while CuL got the highest. This might be related to CuL's distinctive stereochemistry, which may have helped this complex achieve the strongest possible contact with DNA due to its tetragonally deformed octahedral structure. However, the study showed that all the compounds docked are potential against 6LUD and can be selected based on further dynamics, *in-vitro* and *in-vivo* activity studies (Show in Figure 4).

CONCLUSION

The creation of copper (II), nickel (II), and zinc (II) combinations using the dithiocarbamate ligands derived from primaquine phosphate is outlined in our research. There is an urgent need for novel, efficient, secure, and cost-effective cancer treatment options given the rising global resistance to widely used cancer drugs. Complexes of metals built around bioactive ligands and well-known medications have a lot of possibilities as anticancer medications. The periodic table contains a wide range of physical and chemical properties, such as coordination geometry, redox reaction activity, biological compatibility, and anticancer activity. By combining these metal ion capabilities with the ligand's intrinsic bioactivities, it may be possible to enhance the anticancer effects through a variety of routes while perhaps limiting the development of rapid drug resistance. While metal-based anticancer medicines have been the focus of current research, the logical creation of structures of molecules for anticancer action is still largely experimental. The mechanisms of action of metal-based anticancer drugs require further investigation, particularly with regard to cancer-specific targets such DNA interaction DHFR, ergosterol, EGFR, HER2, and tyrosine synthase potential. Advantages in primaquine phosphate compared to the loose ligand were frequently seen after complexing organic compounds with metal ions, although these advantages were not always explained. Organometallic substances like Primaquine complexation exhibited notable anticancer efficacy among the identified metal complexes. Significant anticancer effects were also seen in complexes with ligands comprising thiocarbamate-based Primaquine segments and metal ions like Cu (II), Ni (II), and Zn (II). Overall, this study aims to inspire and guide scientists striving to improve the clinical efficacy of metal-based anticancer medicines. The measured DNA binding values indicate the high DNA-binding affinities of these substances. An adenine hydrogen atom and the oxygen element of the carbonyl compound in the pyrrolidinedione group establish a hydrogen link as the ligand tends to bind in the groove in the minor keys of DNA, according to *in-silico* research. The described chemicals, in conclusion, provide promise as possible picks for anticancer uses. To further assess their potential, plans call for the formulation of nanoparticles and the execution of both *in-vitro* and *in-vivo* studies.

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