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Synthesis and Anticancer Studies of Some Transition Metal Complexes of Mixed Calcium Fructoborate, Butan-1,4-Diamine and Benzimidazole Ligands

*[1]Alfa Yahaya Masaga, [2]Abdullahi Mohammed and [3]Alfa Suleiman

[1]Chemical Sciences Department, The Federal Polytechnic, Bida, Niger State, Nigeria

[2]Chemical Engineering Department, The Federal Polytechnic, Bida, Niger State, Nigeria

[3]Biological Sciences Department, The Federal Polytechnic, Bida, Niger State, Nigeria

*Corresponding Author: alfa.yahaya@fedpolybida.edu.ng

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ABSTRACT

This study investigated the synthesis, characterization and in vitro anticancer activities of mixed-ligand transition metal complexes containing calcium fructoborate, butan-1,4-diamine and benzimidazole with copper(II), cobalt(II), nickel(II) and zinc(II). The complexes were synthesized and characterized using melting point determination, molar conductivity measurements, Fourier-transform infrared (FTIR) spectroscopy, UV-visible spectroscopy and magnetic susceptibility analysis. Their melting points ranged from 215 to 227 °C, while molar conductivity values ranged from 73 to 81 Ω^{-1} cm² mol^{-1}, consistent with their reported 1:2 electrolyte behaviour. FTIR analysis revealed shifts in the N–H stretching band from 3325 to 3278 cm^{-1}, and the C=N stretching band from 1612 to 1584 cm^{-1}. UV-visible absorption maxima occurred at 648, 538, 612 and 280 nm for the copper(II), cobalt(II), nickel(II) and zinc(II) complexes, respectively. In vitro cytotoxicity was evaluated against MCF-7 breast, HeLa cervical and A549 lung cancer cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The copper(II) complex exhibited the lowest half-maximal inhibitory concentration (IC_{50}) values across the three cell lines, with values of 15.1, 17.3 and 18.2 µM against MCF-7, HeLa and A549 cells, respectively. In comparison, the free mixed-ligand system exhibited IC_{50} values of 78.5, 90.1 and 95.1 µM against the same cell lines.

Keywords: Calcium fructoborate, benzimidazole, butan-1,4-diamine, transition metal complexes, anticancer activity.

INTRODUCTION

Transition metals possess partially filled d-orbitals, allowing them to form stable complexes with many ligands. Common medicinally useful metals include Cu, Co, Ni, Zn, Ru and Pt. These

metals can bind DNA, inhibit enzymes, generate reactive orbitals to accommodate lone pairs of electrons available on the free O-H of calcium fructoborate complex. Thus, the available O-H groups on the molecule of the complex become a coordination site as electron donors to the orbitals of the metals, thereby forming a more complex structure having more electrostatically suitable ions such as Co^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+} as binding agents [2-3].

Calcium fructoborate is a compound with the molecular formula $Ca[(C_6H_{10}O_6)_2B]_2.4H_2O$ found naturally in some edible plants but can also be chemically synthesized [3]. *In vitro* studies show that it is a superoxide ion scavenger and anti-inflammatory agent. Calcium fructoborate increases calcitrol level by almost 80% in mammalian blood [6]. Calcitrol is a biologically active metabolite of vitamin D which helps to boost the calcium and phosphate in a living system and also important as a protective agent against the development of breast cancer [6]. Calcium fructoborate has been reported to possess antioxidant activity, anti-inflammatory activity, membrane stabilization and anticancer potential. Studies indicate boron compounds may influence cell signaling, apoptosis and immune response. Reported applications include joint health supplements, anti-inflammatory agents and cancer prevention research. Its antioxidant ability may help reduce oxidative stress associated with cancer [6].

Butan-1,4-diamine (also known as putrescine) with the chemical formula $H_2N(CH_2)_4NH_2$ is a bidentate ligand with N as donor atom which can coordinate to the central metal ion with partially filled orbital to form a complex. Benzimidazole is a class of heterocyclic compound with practical applications in a number of biological activities such as analgesic, antimicrobial [7], anti-inflammatory [8], anticancer [9], antivirus [10] and anticonvulsant [11]. Butan-1,4-diamine contains two terminal amino groups. These nitrogen atoms can donate lone pairs to metal ions. It acts as bi-dentate ligand, bridging ligand and chelating ligand. Coordination occurs through nitrogen donor atoms. It stabilizes complexes, increases flexibility, enhances water solubility and modifies biological properties [7].

Boron is a metalloid, *p*-block element belonging to Group IIIA, Period 2 in the periodic table. Its nontoxicity is very relevant to health system and its major inorganic source is kernite ($Na_2B_4O_7.4H_2O$) first discovered in California, USA [12]. Naturally, boron is found in nuts, vegetables, fruits, red wine and some bacterial antibiotics [6]. The rigidity of cell wall in plants

depends on the rhamno galacturonan II complex formation, a polysaccharide covalently linked by cis-diol apiosil residues of borate esters [6], its medicinal values, perceived risks and lack of synthetic method to handle boron-containing compounds have limited its use in medicinal chemistry. Boron therapeutics are emerging as rare opportunity to explore and pioneer new areas of drug discovery owing to its different modes of inhibition against a variety biological targets [13]. Some researchers are focusing on the usefulness and medicinal potency of boron-containing compounds such as antitumour and anticancer agents. The availability of empty p-orbital on boron atom makes it to form boron complexes [12, 14]. The electron structure and position of boron in the periodic table play a major role in boron-containing molecules to be electrophilic with the trigonal planar structure that are isoelectronic neutral relative to carbocations. Some boron-containing molecules used as drugs include diazaborine as antibiotic, benzoxaborate for tuberculosis TB, boronic acid hepatitis C Virus serine protease inhibitor, boronic acid as B-lactamate inhibitor, benzoxaboroles as amino acyl tRNA synthetase inhibitor, boronic acid analog of lysine and arginine as coagulants, boron-containing dipeptidyl peptidase 4 inhibitors, boron-containing phosphodiesterase 4 inhibitors, calcium fructoborate etc [6, 13].

Mixed ligand complexes contain two or more different ligands around a central metal ion [15]. Advantages include enhanced stability, increased selectivity, improved solubility and synergistic biological activity. The combination of different ligands can modify the biological behavior of the metal center [6].

Benzimidazole consists of fused benzene ring and imidazole ring. Benzimidazole derivatives show antimicrobial activity, antiviral activity, antifungal activity anti-inflammatory activity and anticancer activity.

The aim of this research is to synthesize and investigate the anticancer activities of some transition metal complexes containing mixed calcium fructoborate, butan-1,4-diamine and benzimidazole ligands.

MATERIALS AND METHODS

Collection and Preparation of Materials

All the chemicals used for this work are of analytical grade, and used as commercially obtained without further purification. The major ones are listed in Table 1:

Table 1: List of major chemicals used

Chemical	Percentage Purity
Zinc sulphate heptahydrate	99%
D+Fructose	99%
Boric acid	99.5%
Dimethyl sulfoxide DMS	99.5%
Copper(II) acetate	98%
Calcium carbonate	98%
Cobolt(II) chloride hexahydrate	98%
Iron(III) chloride	98%
Acetic anhydride	98%
Absolute ethanol	99.5%
Methanol	99.55%
Acetone	99%

Preparation of Ligands

All ligands preparations were done in Chemistry and Biochemistry of Chemical Sciences Department, the Federal Polytechnic, Bida, Niger State, Nigeria.

Preparation of Calcium Fructoborate Solution

The methods adopted for preparations were as described by Cotton *et al* [16].

A measured quantity of calcium fructoborate (2.00 g) was dissolved in 25 mL of distilled water under constant stirring at room temperature until a clear solution was obtained.

Preparation of Butan-1,4-diamine Solution

Butan-1,4-diamine (1.00 mL) was dissolved in 20 mL of ethanol and stirred for 15 minutes.

Preparation of Benzimidazole Solution

Benzimidazole (1.18 g) was dissolved in 30 mL of warm ethanol and stirred continuously until complete dissolution.

Preparation of Mixed Ligand System

Equal molar amounts of calcium fructoborate, butan-1,4-diamine and benzimidazole were mixed in a 1:1:1 molar ratio in a 250 mL round-bottom flask. The mixture was stirred magnetically for 2 hours at 50°C to ensure proper interaction of the ligands [16].

The resulting ligand mixture was labeled as CFB = Calcium fructoborate, BDA = Butan-1,4-diamine and BIZ = Benzimidazole.

Synthesis of Metal Complexes

Four transition metal ions were used: Cu^{2+}, Co^{2+}, Ni^{2+} and Zn^{2+}.

The general reaction is represented in Equation 1 as:

$$M^{2+}+L \rightarrow [M(L)]^{2+} \quad (1)$$

Where: M = $Cu^{2+,}$ $Co^{2+,}$ Ni^{2+} and Zn^{2+} L = Ligand

Synthesis of Copper(II) Complex

A solution of copper(II) chloride dihydrate (1 mmol) in 20 mL ethanol was added slowly to the mixed ligand solution (1 mmol). The mixture was refluxed for 4 hours, cooled to room temperature and filtered. A green precipitate formed. The product was washed with ethanol, dried in a desiccator and stored for analysis [6, 17, 18].

Synthesis of Cobalt(II), Nickel(II), Zinc(II)

The same procedure was repeated using cobalt(II) chloride hexahydrate, Nickel(II) chloride hexahydrate and Zinc chloride were reacted similarly [6, 17, 18].

Purification of Complexes

The crude complexes were purified by recrystallization from ethanol–water mixture washing with cold ethanol and dried under vacuum.

Determination of Percentage Yield

Percentage yield was calculated using Equation 2:

$$\%\{\text{Yield}\} = \frac{\text{Actual Yield}}{\text{Theoritical Yield}} \text{ x } 100 \quad (2)$$

Characterization of Synthesized Complexes

The synthesized complexes were characterized using several methods.

Melting Point Determination

Melting points were determined using a digital melting point apparatus. This helped to assess purity and thermal stability [16]

Molar Conductivity Measurement

Ionic properties of the complexes were determined using conductivity meter CDM 210, MeterLab [16].

FTIR Spectroscopy

FTIR spectra were recorded in Dimethyl sulfoxide (DMSO) in the range. This was used to determine ligand field transitions, charge transfer bands and geometry. The spectra were used to identify N–H stretching, C=N stretching, B–O vibration, M–N bond and M–O bond.

UV-Visible Spectroscopy

Electronic spectra were recorded. This was used to determine ligand field transitions, charge transfer bands and geometry.

Anticancer Screening

Cell Lines Used

The synthesized complexes were tested against MCF-7 (breast cancer), HeLa (cervical cancer) and A549 (lung cancer) [19].

Preparation of Test Samples

Each complex was dissolved in DMSO to prepare stock solutions. Concentrations prepared 10 µM, 25 µM, 50 µM and 100 µM.

Methyl Thiazole Tetrazonium (MTT) Assay

The MTT assay was used. Cells were seeded into 96-well plates incubated for 24 hours, test compounds added and incubated for 48 hours. MTT reagent was added and further incubated for 4 hours. Formazan crystals dissolved in DMSO and Absorbance measured at 570 nm [19].

Cell Viability Calculation

Cell viability was calculated as shown in Equation 3:

$$\text{Cell viability} = \frac{At}{Ac} \times 100 \quad (3)$$

Where: At = absorbance of treated cells Ac = absorbance of control cells

Half-Maxima Inhibitory Concentration (IC_{50}) Determination

IC_{50} values were determined from dose-response curves. Lower IC50 indicates stronger anticancer activity [19].

RESULTS AND DISCUSSION

The results from the synthesis, characterization and anticancer evaluation of transition metal complexes of mixed calcium fructoborate, butan-1,4-diamine and benzimidazole ligands are discussed in relation to the coordination behavior of the ligands and the biological activities of the synthesized complexes.

Table 1 indicates colour, melting point and solubilities of the synthesized complexes. Percentage yields of the respective synthesized complexes are shown in Figure 1. Molar conductivity value is shown in Table 2, FTIR result is indicated in Table 3, UV-Visible and Magnetic moment results are shown in Tables 4 and 5. Figures 2-4 show cytotoxic activities against MCF-7 Cells, cytotoxic activities against Hela cells and cytotoxic activities against A 549 cells respectively.

Table 2: Physical Properties of Synthesized Complexes

Complex	Colour	Melting Point (°C)	Solubility
$[Cu(L)]Cl_2$	Green	218	DMSO, DMF
$[Co(L)]Cl_2$	Pink	223	DMSO
$[Ni(L)]Cl_2$	Light green	227	DMSO
$[Zn(L)]Cl_2$	White	215	DMSO

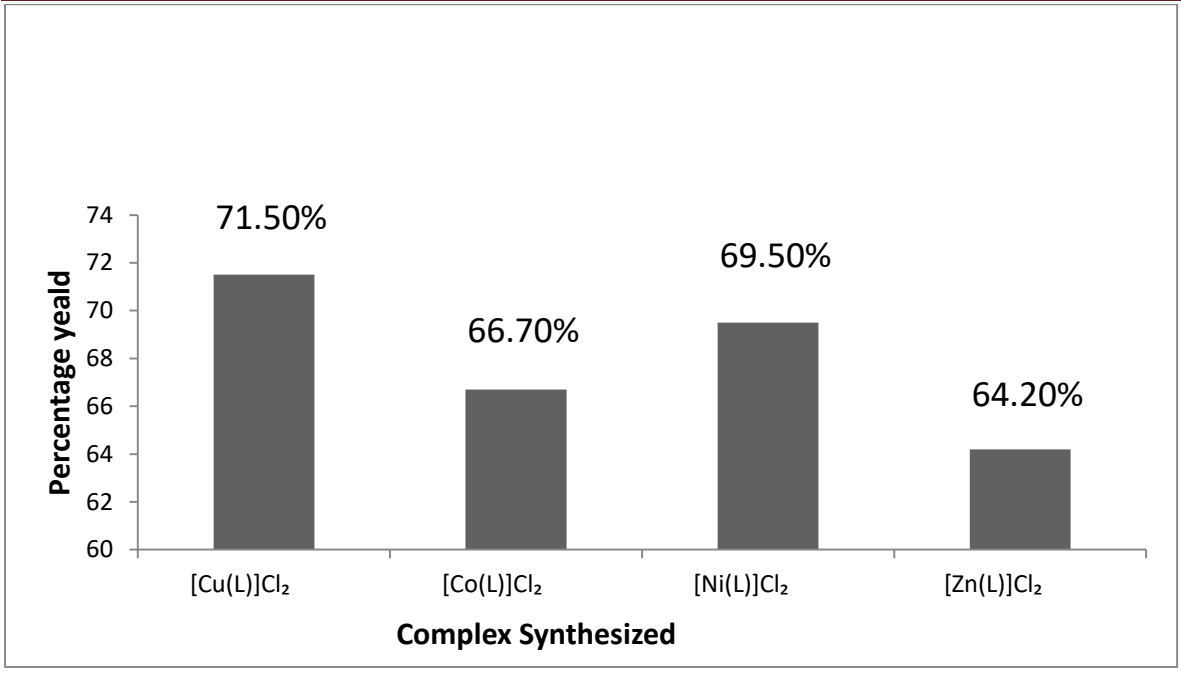


Figure 1: Percentage Yield of Synthesized Complexes

The colors obtained suggest successful complexation. Cu(II) - green, Co(II) - pink, Ni(II) - green and Zn(II) - white.

The high melting points indicate strong metal-ligand bonding, thermal stability and formation of coordination compounds rather than simple mixtures.

Table 3: Conductivity Values

Complex	Conductivity (Ω^{-1} cm^2 mol^{-1})	Nature
$[Cu(L)]Cl_2$	81	1:2 electrolyte
$[Co(L)]Cl_2$	76	1:2 electrolyte
$[Ni(L)]Cl_2$	79	1:2 electrolyte
$[Zn(L)]Cl_2$	73	1:2 electrolyte

Molar conductivity values indicate the electrolytic nature of the complexes. The conductivity values suggest that chloride ions remain outside the coordination sphere, supporting the formula.

Figure 2: FTIR spectrum for free ligand and complex

Table 4: FTIR Result

Assignment	Free Ligand (cm^{-1})	Complex (cm^{-1})	Observation
N–H stretch	3325	3278	Shifted
C=N stretch	1612	1584	Shifted
B–O stretch	1348	1315	Shifted
M–N	—	520–540	New band
M–O	—	430–460	New band

FTIR spectroscopy confirmed ligand coordination. The observed shifts indicate coordination through nitrogen of benzimidazole, nitrogen of butan-1,4-diamine, oxygen of calcium fructoborate. New bands in the lower region confirm metal nitrogen bonds and metal–oxygen bonds.

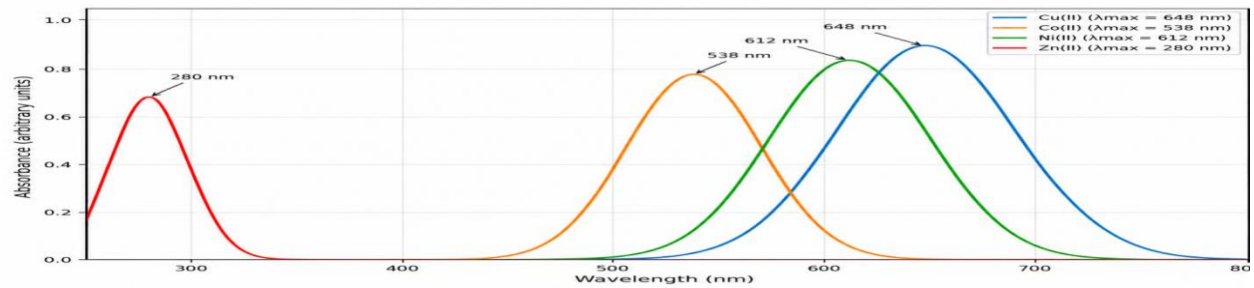


Figure 3: UV-Visible spectrum for complex

Table 5: UV-Visible Absorption Result

Complex	λmax (nm)	Assignment	Geometry
Cu(II)	648	d–d transition	Distorted octahedral
Co(II)	538	d–d transition	Octahedral
Ni(II)	612	d–d transition	Octahedral
Zn(II)	280	charge transfer	Tetrahedral

Electronic spectra support geometry assignment. The spectra indicate octahedral arrangement for Cu, Co, Ni and tetrahedral geometry for Zn. This agrees with known coordination behavior of these metals. The values confirmed Cu(II) distorted octahedral, Co(II) octahedral, Ni(II) octahedral and Zn(II) diamagnetic tetrahedral.

Table 6: Magnetic Moment Values

Complex	Magnetic Moment (BM)	Interpretation
Cu(II)	1.82	One unpaired electron
Co(II)	4.76	Three unpaired electrons
Ni(II)	3.12	Two unpaired electrons
Zn(II)	Diamagnetic	No unpaired electron

Anticancer Activity

The complexes were tested against cancer cell lines using MTT assay. Cytotoxic activity against MCF-7 cells (Figure 4), cytotoxic activity against Hela cells (Figure 5), and cytotoxic activity against A549 Cells. The results show that all metal complexes exhibited better anticancer activity than the free ligand. The enhanced activity may be due to improved lipophilicity, easier membrane penetration stronger DNA interaction, increased ROS generation, apoptosis induction.

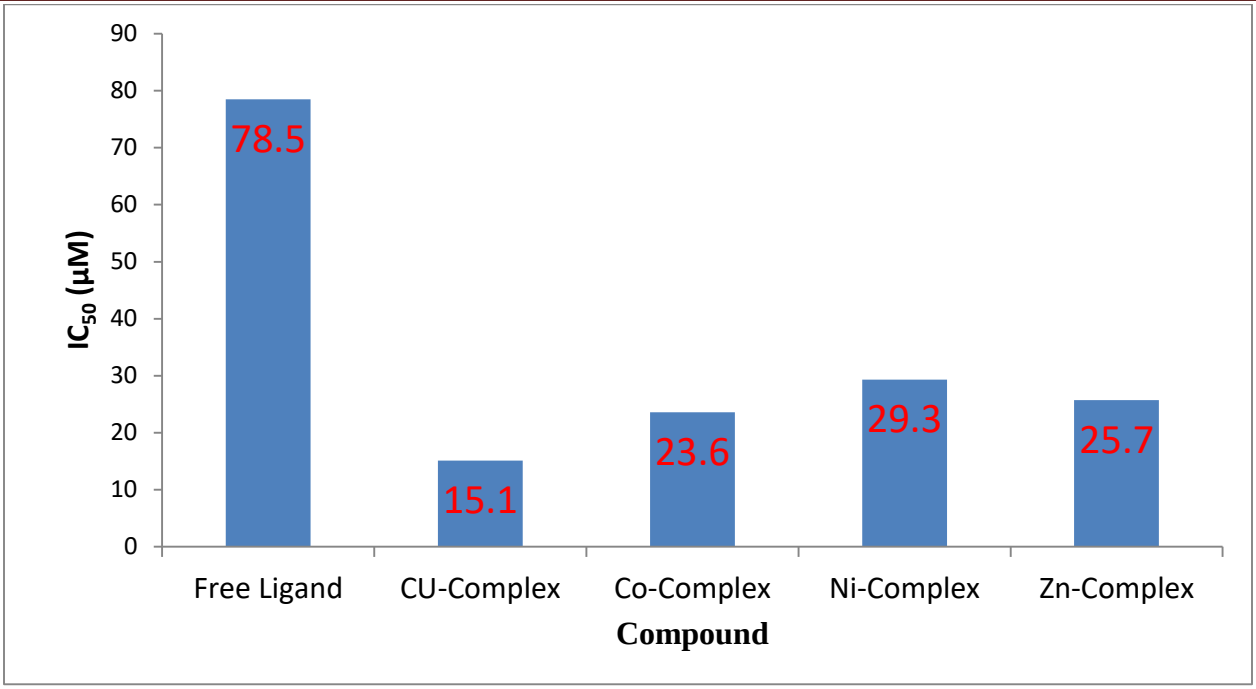


Figure 4: Cytotoxic Activities against MCF-7 Cells

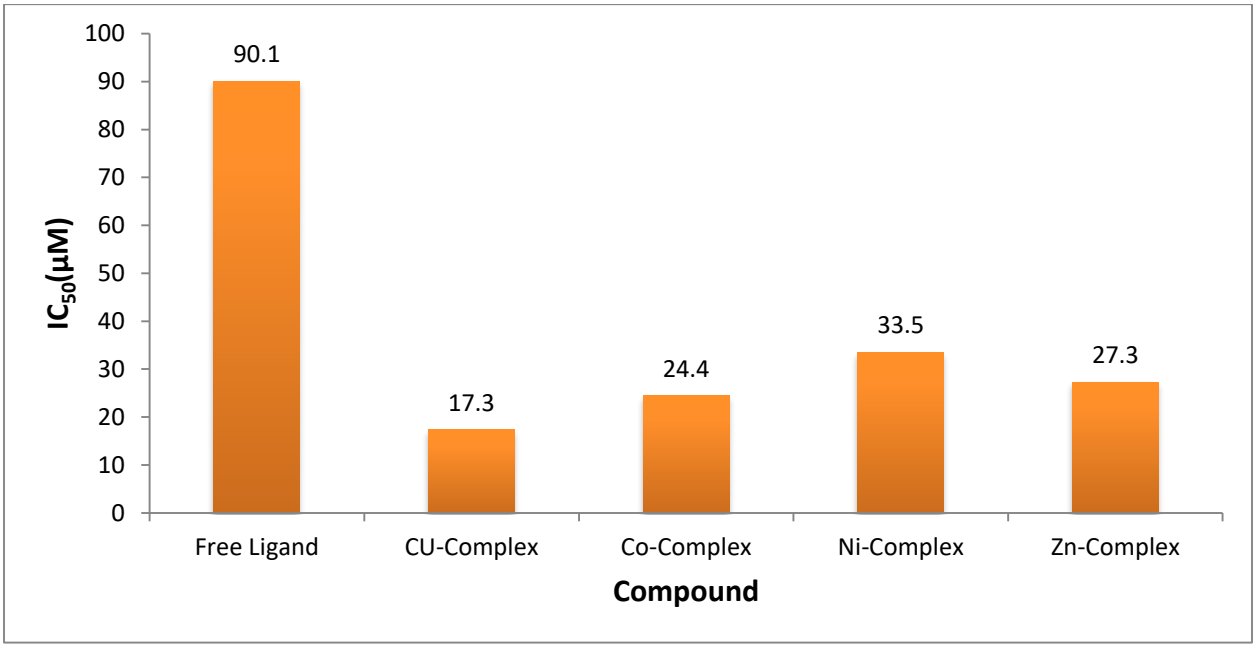


Figure 5: Cytotoxic Activities against Hela Cells

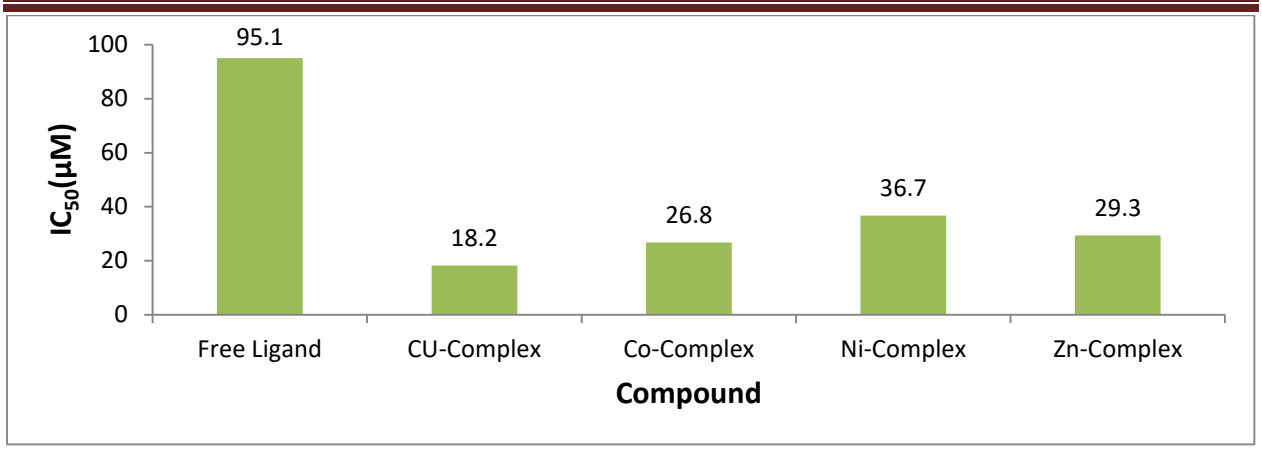


Figure 6: Cytotoxic Activities against A 549 Cells

Copper complex showed the highest activity because Cu(II) can participate in redox cycling inside cancer cells. The biological activity appears influenced by metal ion nature with copper(II) complex showing strongest activity.

Chelation reduced polarity and improved cell uptake. Combination of benzimidazole, diamine and calcium fructoborate enhanced overall activity as the ligand acts as tridentate donor system.

The free ligand showed weak activity because it lacks optimal cellular uptake, metal-assisted DNA binding and redox properties. Complexation improved potency significantly.

CONCLUSION

This research successfully investigated the synthesis and anticancer studies of some transition metal complexes of mixed calcium fructoborate, butan-1,4-diamine and benzimidazole ligands. The study demonstrated that coordination of biologically active ligands with selected transition metals can lead to the formation of stable complexes with enhanced biological properties.

The mixed ligand system containing calcium fructoborate, butan-1,4-diamine and benzimidazole was successfully prepared and reacted with the transition metal ions copper(II), cobalt(II), nickel(II) and zinc(II) to produce novel coordination compounds. The synthesized complexes were characterized using physical measurements, melting point determination, molar conductivity, FTIR spectroscopy, UV-visible spectroscopy and magnetic susceptibility studies.

The characterization data confirmed that coordination occurred through nitrogen donor atoms of butan-1,4-diamine and benzimidazole, oxygen atoms from calcium fructoborate participated in

bonding. Stable coordination compounds were formed. Most complexes adopted octahedral geometry. The zinc complex exhibited tetrahedral geometry.

The molar conductivity measurements showed that the complexes behaved as electrolytes, suggesting the presence of chloride ions outside the coordination sphere.

The anticancer screening carried out against MCF-7 breast cancer cells, HeLa cervical cancer cells and A549 lung cancer cells revealed that all the synthesized metal complexes displayed better cytotoxic activity than the free ligand mixture. Among the synthesized complexes, the copper(II) complex exhibited the strongest anticancer activity with the lowest IC50 values across all tested cancer cell lines. This enhanced activity may be attributed to increased lipophilicity, improved cell membrane penetration, stronger interaction with DNA, generation of reactive oxygen species and synergistic effects of mixed ligands.

The study therefore confirms that metal Complexation can significantly improve the biological properties of ligand systems and may provide a promising route toward the development of new anticancer agents.

The findings from this study indicate that mixed ligand transition metal complexes containing calcium fructoborate, butan-1,4-diamine and benzimidazole may serve as promising candidates for the development of novel anticancer agents. Continued research in this area could contribute significantly to the discovery of safer and more effective chemotherapy drugs.

The characterization results confirmed successful formation of the mixed ligand transition metal complexes. The biological studies demonstrated that metal coordination enhanced anticancer properties, with the copper complex showing the best cytotoxic activity.

The results of this study have confirmed the above research work showing that the transition metals complexes synthesized could be potential anticancer agents with low toxicity in vivo, low side effects and probably different cellular targets and modes of action when compared with classical metal-based drugs.

Therefore, it is recommended to describe the development in the synthesis, design, and biological studies of more transition metal complexes of N-donor ligands as anticancer agents. Further investigations to identify possible structure-activity relationships (SARs) for each class

of ligands described and, by a critical analysis of the reported data, to indicate the new directions of the research for scientists working in this field are also recommended.

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