



Original Article

Synthesis, Spectral, Cancer Inhibitory Activity and Antimicrobial Studies of Cobalt(II) and Nickel(II) Metal Complexes Containing Azo Derived from 2-Amino Benzimidazole

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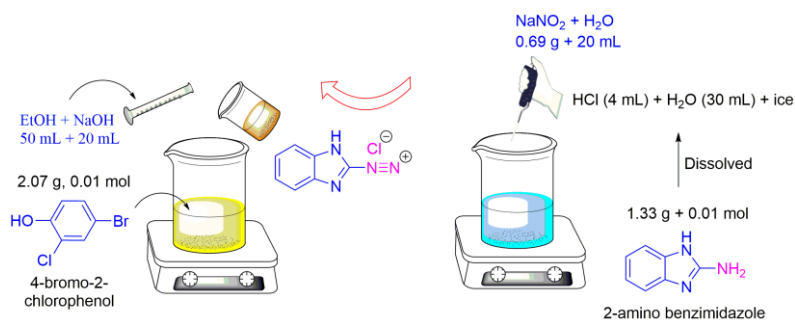
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ABSTRACT

The production of the tridentate Azo ligand 2-[2-(benzimidazolyl)azo]-4-bromo-6-chloro phenol (BIABrCIP) complexes using Co(III) and Ni(II) is discussed in this article. Spectral methods including ¹H-NMR, UV-Vis, FT-IR, magnetic moment measurements, molar conductance, and elemental analysis were used to thoroughly explore the coordination mode of azo ligand containing NNO donor atoms with metal ions. The octahedral geometry for the synthesized Ni(II) and Co(III) complexes was suggested from the analytical and spectral studies. According to *in vitro* assays for antibacterial activity, the majority of produced compounds exhibit good action against a variety of microorganisms, including *Candida albicans*, *Staphylococcus aureus*, and *Escherichia coli*.

GRAPHICAL ABSTRACT



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Introduction

Azo compounds have several purpose and noteworthy activities; it is the generality utilizing dyes [1]. "They are applied to die many substances, they are a highly colored category of chemical compounds utilized as dyes for a long time, especially fabrics and they are attracting concern in scientific research. "It is widely distributed in nature and interferes with the synthesis of many biomolecules, vitamins, dyes, and enzymes. In this work; heterocyclic amine compound was prepared and widely used in medicine and pharmacology [2, 3]. Imidazole derivatives play important roles in industrial and medical fields such as metronidazole, medetomidine, and ornidazole used in surgery and pain relief. Antibacterial benzimidazoles are very useful compounds in biochemical research. This is evident in this compound in the benzimidazole nucleus: riboglitazone as antidiabetic, dabigatran as anticoagulant, candesartan as antihypertensive, marivavir as antiviral, flibanserin drug for HSDD Telmisartan as a drug [4-6]. Benzimidazole derivatives have biological activities such as antineoplastic, cardiostimulants, anticonvulsant, and antidepressant [7, 8].

Materials and Methods

Organic solvents and chemicals were of analytically pure grade and obtained from B.D.H, Sigma Aldrich, and Fluka. UV/vis spectra were recorded by Shimadzu UV-Vis 1700 spectrometer in 200-1000 nm range at room temperature in absolute ethanol as a solvent (10^{-3} M), FT-IR spectra were recorded with Shimadzu FT-IR-4800S infrared spectrometer by using KBr disk. $^1\text{H-NMR}$ studies were performed via (BRUKER 400MHz) spectrometer (Germany) using DMSO-d_6 as the solvent, and TMS as the internal standard at the Isfahan University, Iran. Mass spectra was studied with Shimadzu Ufms Gcms-TQ 8030 and Digital Conductivity meter-WT-720-inolab (Germany) was used to measure the electrical conductivity the melting points were measured for ligand and complexes via electro thermal melting point 9300. The ligand and its complexes were imaged using scanning electron microscopy

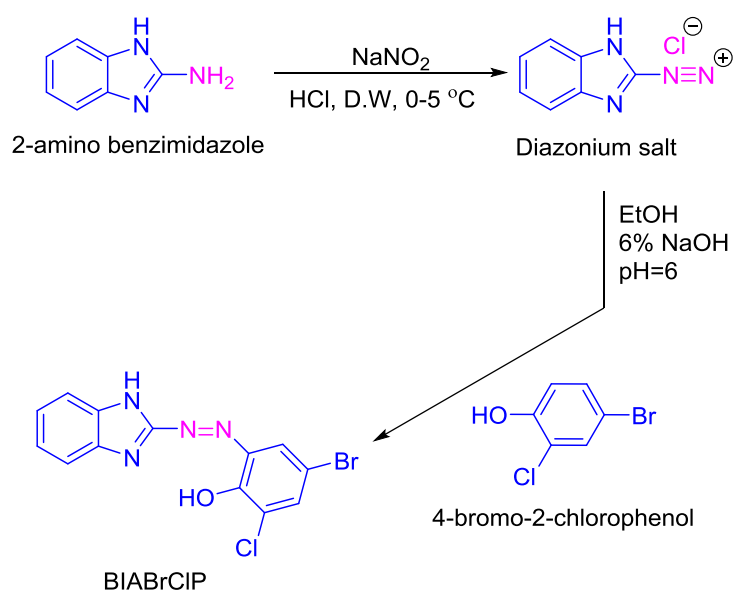
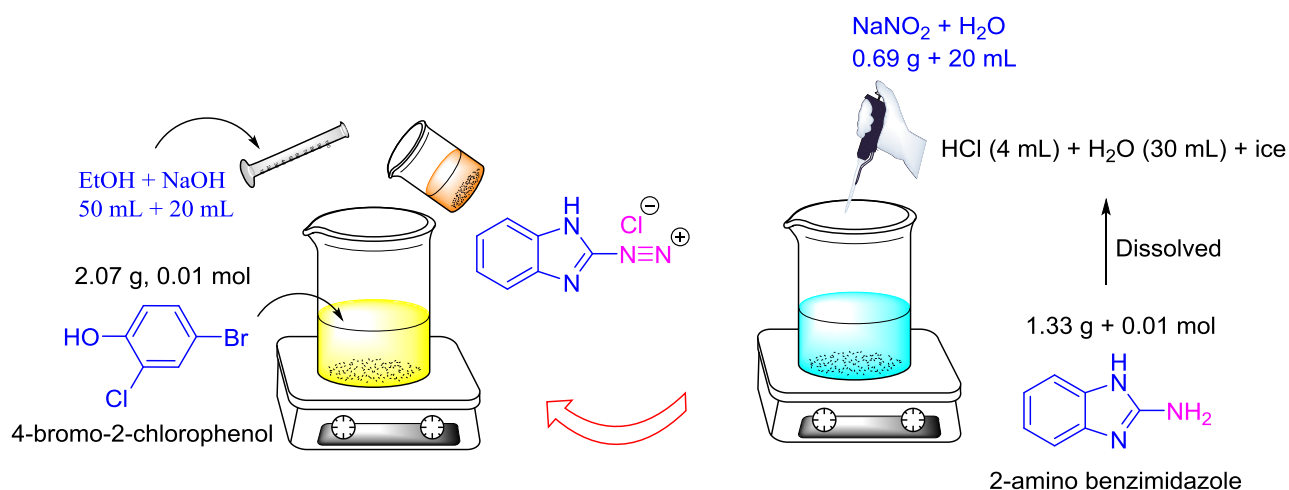
(SEM) on the micrograph Kyky 3200. This ligand and its complexes were subjected to thermo gravimetric studies using the Perkin Elmer TGA-4000, which revealed PL-TG. Relying on a micro analytical unit from Eurovector, EA300A, Italy, elemental analysis (C, H, and N) was carried out. Balance Magnetic (MSB-MKI) equipment was used to calculate the produced complexes' magnetic susceptibility.

Synthesis of the azo ligand 2-[2'-(benzimidazolyl)azo]-4-bromo-6-chloro phenol (BIABrCIP)

2-Aminobenzimidazole (1.33 g, 0.01 mol) dissolved in a mixture of (30 mL D.W + and 4 mL of concentrated HCl) and stirred in ice bath, a NaNO_2 solution (0.689 g, 0.01 mol, 20 mL H_2O) was added dropwise into the solution, and stirred the mixture for 15 min at 0-5 °C. The resulting diazonium chloride solution was added dropwise to the mix. containe 4-bromo-2-chloro phenol (2.07 g, 0.01 mL) and (10% NaOH in 50 mL ethanol) with constant stirring at 0.0-5.0 °C and allowed to stand overnight and acidified with dilute HCl to pH=6., orange-red colored solution, Darkness is observed. The crystals were filtered off, washed with D.W and purified by recrystallization from hot ethanol and dried at room temperature [9]. The structural of the ligand (BIABrCIP) was shown in [Scheme 1](#).

Synthesis of metal complexes

The solutions of the Cobalt(II) chloride hexahydrate salts and $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (0.1290 g, 0.0010 mol) and the azo ligand was refluxed for 1 hour. The complexes of cobalt(III) and nickel(II) were prepared by dissolving (0.703 g, 0.0020 mol) of ligand (BIABrCIP) in 50 mL of ethanol which then added dropwise with vigorous stirring to $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (0.1298 g, 0.001 mol) and $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (0.1296 g, 0.001 mol) dissolved in 10 mL of hot distilled water. The mixture was heated to 60 °C for 35 min, then left over night. The solid complex formed was removed by filtration, washed with 10 mL hot ethanol until the filtrate becomes colorless and dried in a vacuum desiccator over anhydrous CaCl_2 (yields = 75-85%).



Scheme 1: Preparation of the Ligand (BIABrCIP)

Results and Discussion

Using the MTT assay, the cytotoxicity of the Ni(II)-complex was investigated in this study on human (MCF-7) cancer cells as well as normal cells. With an IC_{50} value of 130.3 $\mu\text{g}/\text{mL}$ against cancer cell lines, the Ni(II)-complex demonstrated selective cytotoxicity, whilst its IC_{50} value of 7994 $\mu\text{g}/\text{mL}$ indicated that it was very safe for use with normal cell lines in humans. Doubtless, the findings point to the potential for their use as anticancer medications in the treatment of breast cancer in

the fields of medicine and pharmacy. Table 1 lists the analytical values for the ligand (BIABrCIP) and its metal complexes along with a description of some of their physical properties.

The colored solids that are stable at room temperature make up each and every metal complex. All of the complexes are insoluble in water and freely soluble in DMSO, DMF CHCl_3 , methanol, and ethanol. The ligand (BIABrCIP) is soluble in the majority of organic solvents. Metal complexes show 1:2 (metal-ligand) stoichiometry.

Table 1: Analytical and physical characterization data of the ligand (BIABrCIP) and their complexes

Compound	m.p (°C)	Yield (%)	M.f (M.wt)	Found (Calc.)%			
				C%	H%	N%	M%
Ligand =(BIABrClP)	189	83%	C ₁₃ H ₈ BrClN ₄ O 351.59	(44.41) 43.74	(2.29) 2.18	(15.93) 14.98	.
[Co(BIABrClP) ₂] Cl.H ₂ O	264	75%	C ₂₆ H ₁₆ Br ₂ Cl ₃ CoN ₈ O ₃ 813.56	(38.38) 37.92	(1.98) 1.87	(13.77) 13.12	(7.24) 6.89
[Ni(BIABrClP) ₂].H ₂ O	242	78%	C ₂₆ H ₁₆ Br ₂ Cl ₂ NiN ₈ O ₃ 777.87	(40.15) 40.00	(2.07) 2.11	(14.40) 13.75	(7.54) 7.10

¹H-NMR spectra

The ¹H-NMR spectra of the ligand (BIABrClP) in Figure 1 was recorded in DMSO-*d*₆ as solvent and TMS as an internal reference and the assignment has been made on the basis of spin-spin interaction. The ¹H-NMR spectrum exhibited the singlet and multiplet signals at about δ=6.832-8.004 ppm assigned to phenyl-CH and benzimidazole-CH protons, signal at δ=11.9 ppm due to (N-H) of the imidazole ring [10], a signal at δ=10.01 ppm due to O-H of the hydroxyl group, and a signal at δ=2.30 ppm due to the solvent proton.

Infrared spectra

The spectrum of the free ligand (BIABrClP) shown in Figure 2 showed a broad and medium-intensity absorption band at a frequency of 3630.180 cm⁻¹ attributed to the phenolic ring hydroxyl group. In

addition, the spectrum of the free ligand revealed a strong band at the frequency 3440.77 cm⁻¹ due to the stretchy vibrations of the N-H bond of the benzimidazole ring and the lack of change of this band in all the spectrums of the complexes, indicating that the NH bond did not participate in the complexing process or complex formation [11]. The spectrum of all complexes indicates a wide absorption band at frequency 3362.82-3423.39 cm⁻¹ that belongs to the O-H ν bond of water molecules in these complexes, illustrating the presence of a water molecule in each of the complexes [12]. As for the weak band at frequency 3111.82 cm⁻¹, in the free ligand spectrum, it goes back to the stretch frequency of the aromatic ν (C-H) bond, and this band is fixed in intensity and position for both the ligand spectrum and the spectra of complexes, indicating that it is not affected by the coordination process due to its distance from the coordination sites.

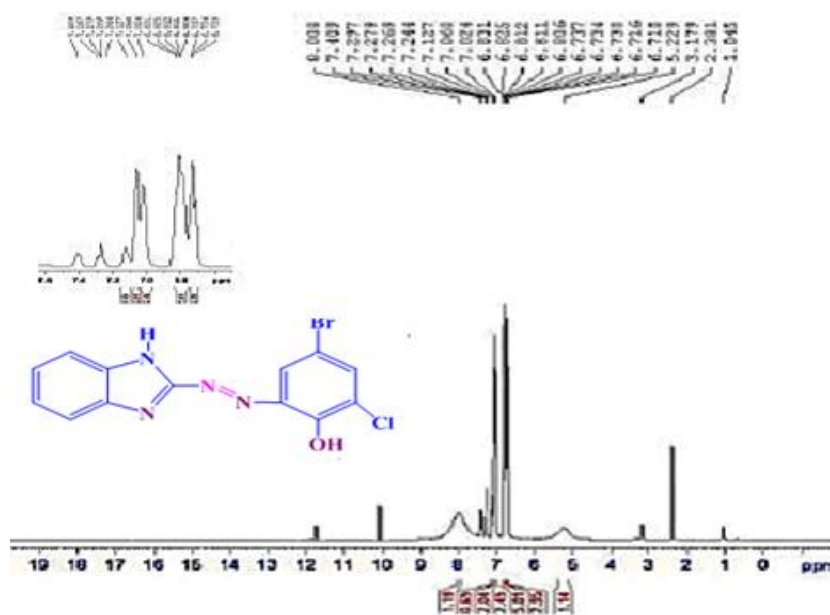
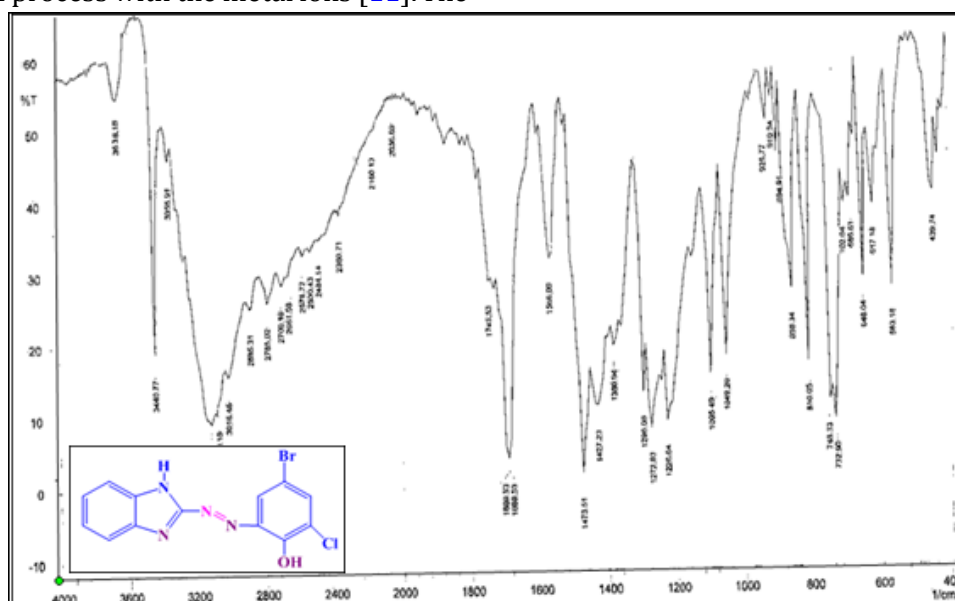


Figure 1: ¹H-NMR spectrum of ligand (BIABrClP)

The spectrum of the free ligand showed a strong absorption band at the frequency of 1688.53 cm^{-1} due to the stretch frequency of the ν (C=N) bond of the benzimidazole ring [13]. The nitrogen of the benzimidazole ring (N_3) in the process of coordination with metal ions. The appearance of a band at a frequency of 1509.09 cm^{-1} in the spectrum of the free ligand due to the azo bridge group shifted to a lower frequency when comparing the spectrum of the ligand with the spectra of the metal complexes, and this is an indication that the azo group participated in the coordination process with the metal ions [11]. The

spectrum of the ligand also showed a band at the frequency of 1390.04 cm^{-1} belongs to the C=C ν band, while the absorption band in the 1272.93 cm^{-1} region is due to the stretchy frequency of the C-N = N-C bond. Nitrogen of the azo-bridge group far from the benzimidazole ring. For all metallic complexes, a new band appears in the far region of the IR spectrum at frequencies $426\text{-}530\text{ cm}^{-1}$ and it is not present in the spectrum of the free ligand. Oxygen and nitrogen with a metal ion. Figures 3 and 4 depict the spectra of its complexes spectra.



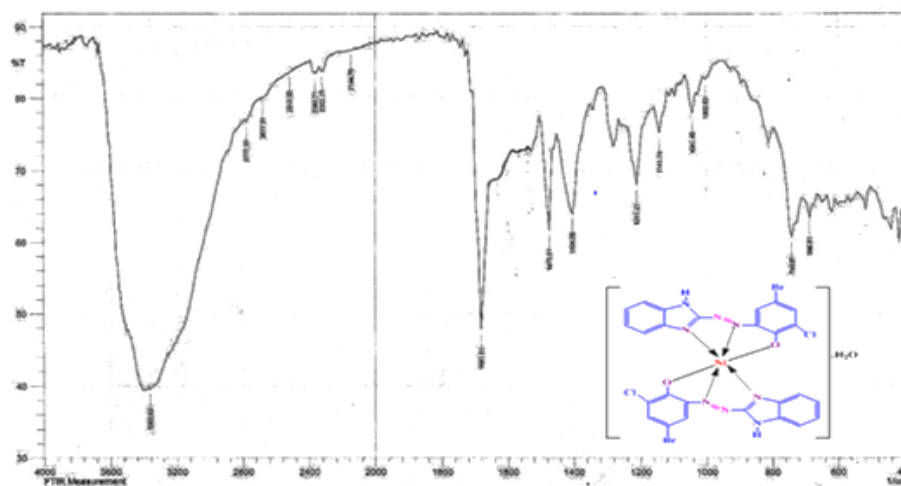


Figure 4: FT-IR Spectra of Ni complex

UV-vis absorption spectra

The spectrum of the free ligand (BIABrCIP) gave three peaks, the first at 418 nm (23923 cm⁻¹) (following the electron transfer ($\pi \rightarrow \pi^*$) of the azo group (N=N), where a red shift appears due to the interaction between the ligand and the metal ion. The second peak appeared at 315 nm (31746 cm⁻¹) belongs to the electronegativity ($\pi \rightarrow \pi^*$) of the (C=C) bond in the benzimidazole and the aromatic ring, the third peak at 285 nm (35088 cm⁻¹) belongs to the ($n \rightarrow \pi^*$) electron transfer of the hydroxyl phenol ring [14].

The absorption region bands assignment and the proposed geometry of the ligand (BIABrCIP) and its metal complexes are listed in Table 3. The UV-Visible spectra of the ligand (BIABrCIP) and its metal complexes are shown in Figures 5, 6, and 7.

The electron spectrum of the cobalt(III) complex showed two absorption peaks, one at 575 nm (17391) cm⁻¹ and the other at 515 nm (19417) cm⁻¹,

which is due to the allowed electron transitions $^1A_{2g} \rightarrow ^1T_{1g}(F)(\nu_1)$ and $^1A_{2g} \rightarrow ^1T_{2g}(\nu_1)$, respectively [15].

The electronic spectrum of the Nickel(II) complex showed three absorption peaks at 920 nm (10869) cm⁻¹, 588 nm (17007) cm⁻¹, and 485 nm (20618) cm⁻¹ following the electron transitions $^3A_{2g} \rightarrow ^3T_{2g}(F)(\nu_1)$, $^3A_{2g} \rightarrow ^3T_{1g}(F)(\nu_2)$, and $^3A_{2g} \rightarrow ^3T_{1g}(p)(\nu_3)$, respectively [16]. The magnetic moment of the complex is 2.81 B.M. Therefore, octahedral stereochemistry has been suggested.

Molar Conductance measurements

The molar conductances of Co(III) and Ni(II) complexes in ethanol, DMF, and s. cm². mol., respectively, are 39.98, 7.65, 71.84, and 19.87 at laboratory temperature. To calculate chloride ions, the Mohr Method (17) was applied. The Ni(II)-complex's low molar conductance value, which falls within the range of Ni(II)-complexes that are not electrolytes, as indicated in Table 4.

Table 3: Electronic spectra (nm and cm⁻¹), electronic transition, and magnetic moments of the ligand (BIABrCIP) and its metal complex

Complexes	λ_{max} (nm)	Absorption bands (cm ⁻¹)	Transition	μ_{eff} (B.M)
HL ₁ =BIABrCIP (BIABrCIP)	418	23923	$\pi \rightarrow \pi^*$	-
	315	31746	$\pi \rightarrow \pi^*$	
	285	35088	$n \rightarrow \pi^*$	
[Co(BIABrCIP) ₂]Cl.H ₂ O	575	17391	$^1A_{2g} \rightarrow ^1T_{1g}(F)(\nu_1)$	Dia
	515	19417	$^1A_{2g} \rightarrow ^1T_{2g}(F)(\nu_2)$	
[Ni(BIABrCIP) ₂].H ₂ O	920	10869	$^3A_{2g} \rightarrow ^3T_{2g}(F)$	2.81
	588	17007	$^3A_{2g} \rightarrow ^3T_{1g}(F)$	
	485	20618	$^3A_{2g} \rightarrow ^3T_{1g}(p)$	

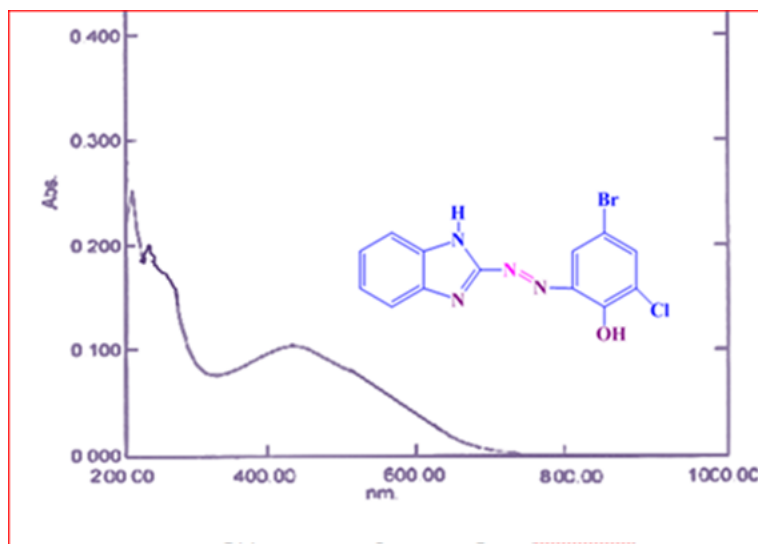


Figure 5: UV-Vis spectra of ligand (BIABrClP)

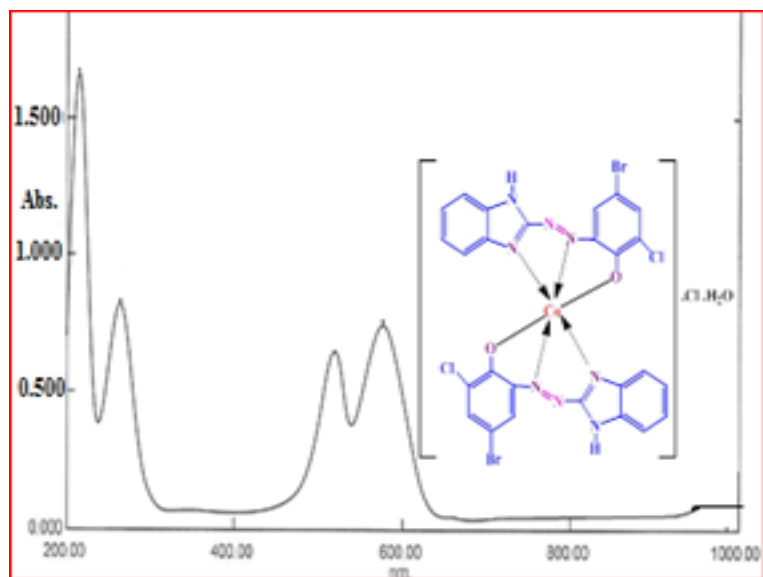


Figure 6: UV-Vis spectra of [Co(BIABrClP)₂]Cl.H₂O

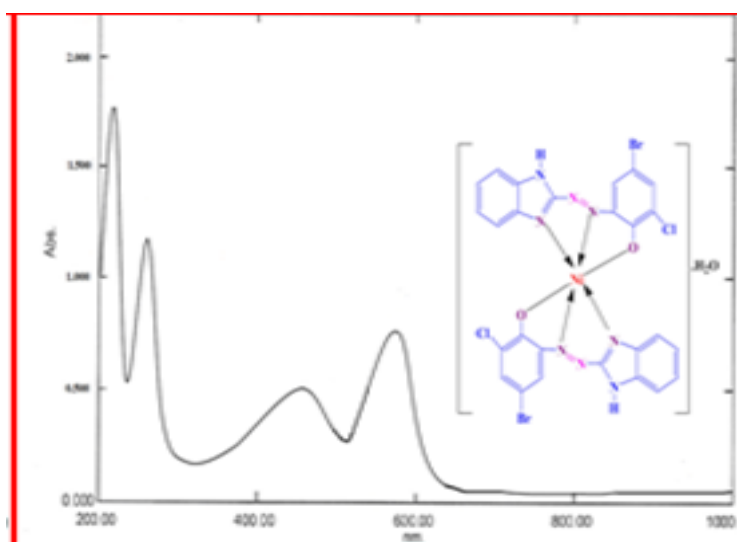


Figure 7: UV-Vis spectra of [Ni(BIABrClP)₂].H₂O

No ions exist outside of the coordination sphere as a result, and the Co(III) complex behaves as 1:2 electrolytes, which suggests that they are ions. White precipitates formed when silver nitrite in aqueous solution was added to the complex of

cobalt (III), indicating the presence of chloride ions beyond the coordination sphere. The structural formulae of that very ligand and its metal complexes can be suggested in Figure 8.

Table 4: Molar conductivity (ϵ) values of metal complexes solutions of ligand in ethanol and DMF at concentration (1×10^{-3}) in laboratory temperature

Metal ion	Molar conductivity(S/m)	
	EtOH	DMF
[Co(BIABrCIP) ₂].Cl.H ₂ O	39.98	71.84
[Ni(BIABrCIP) ₂].H ₂ O	7.65	19.87

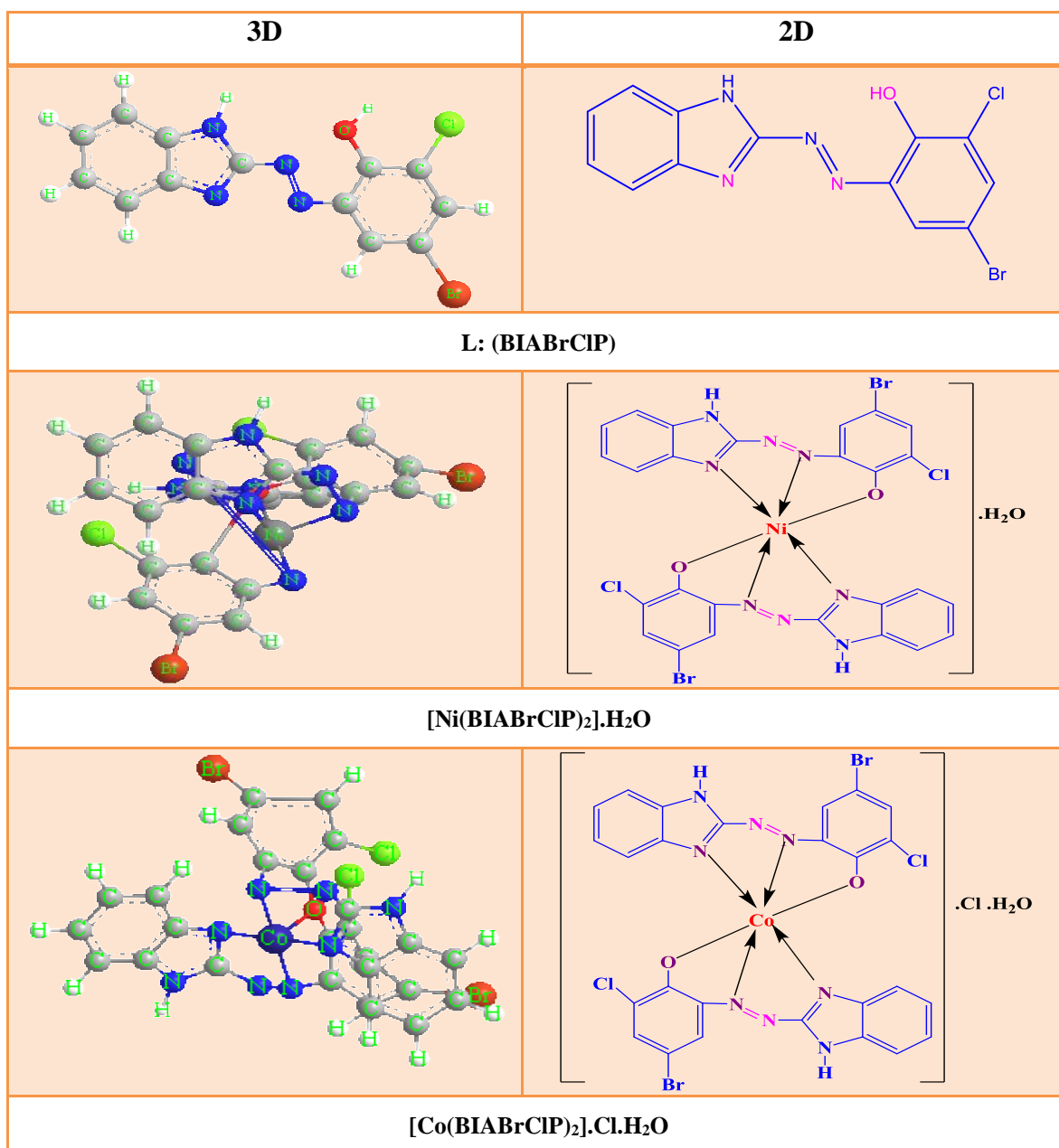


Figure 8: Proposed structure formula of the free ligand and metal complexes

Antimicrobial studies

The antimicrobial activity of the ligand (BIABrCIP), Co(III), and Ni(II) complexes were studied against two pathogenic bacterial strains, one gram negative (*Escherichia Coli*) and one gram positive (*Staphylococcus Aureus*) bacteria and one fungal strain (*Candida Albicans*), as depicted in Figure 9 and 10. The blank test (solvent) DMSO did not show any inhibition zones, as presented in Table 5. Antibacterial effects of Co(III) complex was more significant than those of the remaining compounds. The ligand and metal complexes exhibits higher antifungal effectiveness against *Candida Albicans*. Moreover, the antimicrobial capability has been assessed with the comparison of dicloxacillin standard antibiotic drug and Ketoconazole standard antifungal drug [18].

Cytotoxic studies - MTT assay

In the current study, the cytotoxicity of $[\text{Ni}(\text{BIABrCIP})_2] \text{Cl} \cdot \text{H}_2\text{O}$ complex toward breast cancer cell lines was investigated by MTT test, where the breast cancer viability cells was 29.44% at 400 $\mu\text{g}/\text{mL}$ and viability for normal cells was 75.64 % at the same concentration [19]. Table 6 and Figure 11 show the effect of Ni(II) complex on breast cancer (MCF-7) cell and it compared with normal cell lines at the same concentration under 37 °C. The (IC_{50}) of cancerous cells was 130.3 $\mu\text{g}/\text{mL}$, while it is 7994 $\mu\text{g}/\text{mL}$ for normal cells. The results explain the possibility to use the Ni(II) complex as the treatment of some human cancers. After addition some modifications.

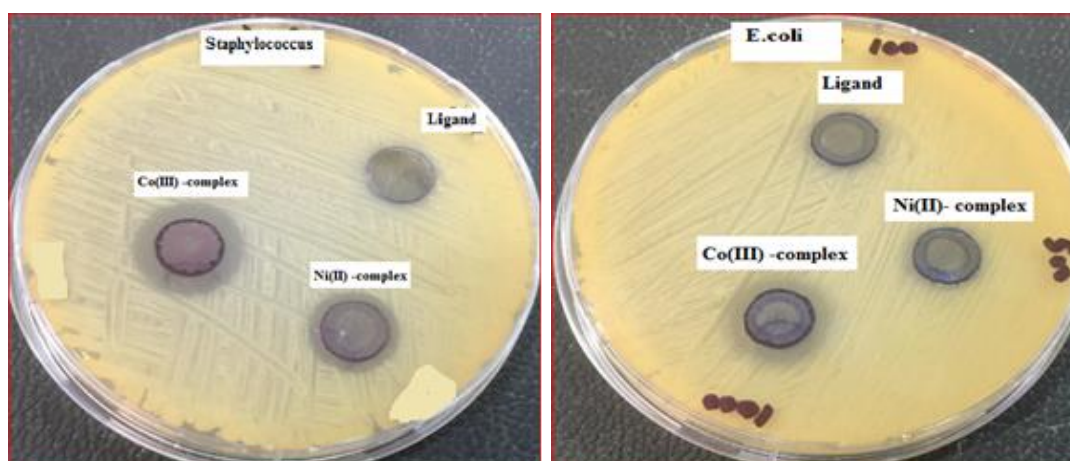


Figure 9: Effect of ligand (BIABrCIP) and its prepared metal complexes on (*Escherichia Coli* & *staphylococcus*) bacteria growth

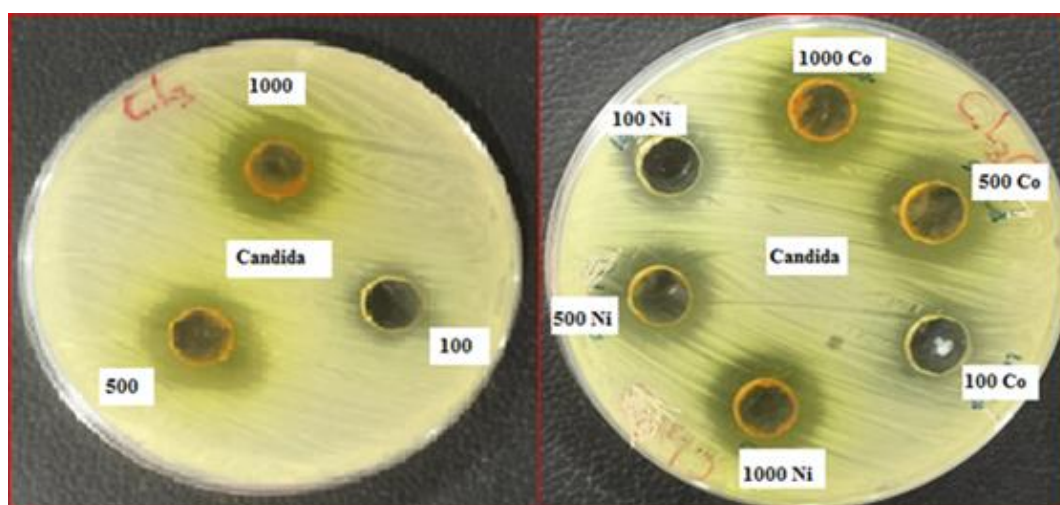


Figure 10: Effect of ligand (BIABrCIP) and its prepared metal complexes on fungal *Candida* growth

Table 5: Antimicrobial activity data of the ligand and its metal complexes dicloxacillin and Ketoconazole are drugs of reference

Compound	Anti-bacterial Activity		Anti-fungal Activity
	Gram-Positive <i>Staphylococcus aureus</i>	Gram-Negative <i>Escherichia coli</i>	<i>Candida</i>
DMSO(Solvent)	-	-	-
Dicloxacillin	-	+
Ketoconazole	-
L: (BIABrCIP)	-	+	+++
[Ni(BIABrCIP) ₂].H ₂ O	++	++	+++
[Co(BIABrCIP) ₂].Cl.H ₂ O	+++	+++	+++

Table 6: Effect of [Ni(BIABrCIP)₂].Cl.H₂O on breast cancer cells and normal cells

Con. (µg.mL ⁻¹)	Mean Percentage (%) for each cell line			
	[Ni(BIABrCIP) ₂].Cl.H ₂ O			
	line cells of breast	MCF-7 (Cancerous)	line cells of breast	WRI-68 (Normal)
	Cell Viability	Cell Inhibition	Cell Viability	Cell Inhibition
6.25	96.34	3.66	95.95	4.05
12.5	96.10	3.9	94.41	5.95
25	95.20	4.8	93.58	6.42
50	90.11	9.89	93.10	6.9
100	60.44	39.56	90.24	9.76
200	55.02	44.98	80.46	19.54
400	29.44	70.56	75.64	24.36

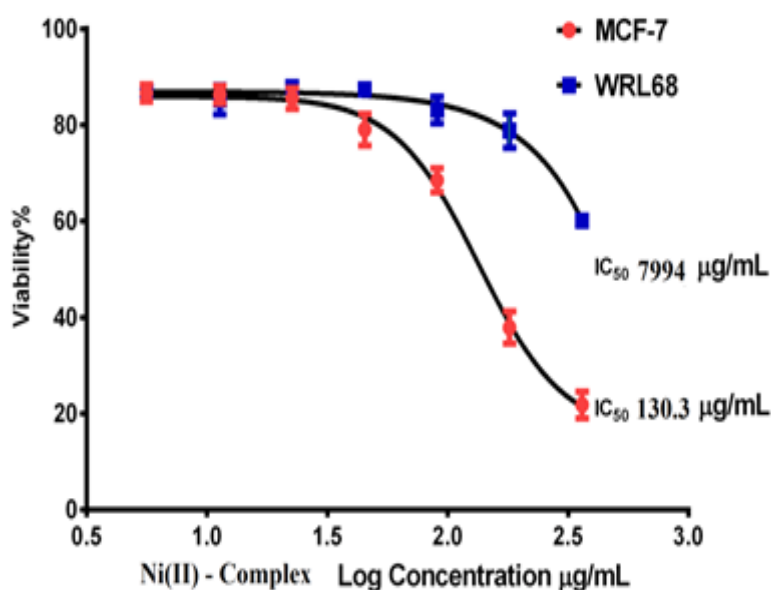


Figure 11: IC₅₀ µg/mL value for the complex

Conclusion

In the current study, the complexes [Co(BIABrCIP)₂].Cl.H₂O and [Ni(BIABrCIP)₂].Cl.H₂O of the Azo ligand were examined. In the complexes' proposed structural formula, the ligand

(BIABrCIP) is depicted as behaving as a tridentate ligand once it has been coordinated with Co(III) and Ni(II) ions. Against Gram-positive and Gram-negative bacteria, the biological effects of azo ligand and metal complexes are examined. A few of the complexes were shown to have a significant amount of activity toward some of the species.

Escherichia coli and *Staphylococcus aureus* are somewhat resistant to the investigated compounds' antibacterial effects, but *Candida albicans* is well inhibited by their antifungal properties. In addition, [Ni(BIABrCIP)₂]'s anticancer effectiveness. Using the breast cancer cell line MCF-7 as a test subject, cytotoxicity activity of H₂O was investigated.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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