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Synthesis, Characterization and Biochemical Activity of Macrocyclic Complexes of Co (II) metal

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ABSTRACT

Some novel Co(II) macrocyclic complexes have been prepared by template condensation of bis(benzil)4-bromo 1,2-phenylenediamine with appropriate diamine i.e. 4-bromo 1,2-phenylenediamine, 4-chloro1,2-phenylenediamine, 4-nitro1,2-phenylenediamine, 1,2-diaminotoluene, 1,2- diaminopropane and ethylenediamine in the presence of Co(II) metal ion to form complexes. The complexes have been identified through the use of physicochemical and spectroscopic methods viz. IR, ^{1}H NMR and Mass. This research has led to the suggestion of an octahedral geometry around the central metal ion. Antifungal and antibacterial activities of the recently synthesized ligand and its metal derivatives have been investigated. In the current study, the synthesized ligand and metal complexes were tested for their cytotoxic properties against two human cancer cell lines breast cancer (MCF-7) and cervical cancer (HeLa) using MTT cell viability assay. Moreover, the cytotoxicity of the synthesized Co (II) complex was tested on human normal embryonic kidney cell line (HEK293). The Co (II) complex [$C_{40}H_{26}BrCl_2CoN_5O_2$] excelled in halting the proliferation of the cervical cancer cells (HeLa) with median inhibitory concentration (IC_{50}) value of 88.45 µM. The complex, [$C_{40}H_{26}Br_2Cl_2CoN_4$] showed selective cytotoxicity against breast cancer cell line MCF-7 with IC_{50}= 85.88 µM. whereas, it displayed insignificant cytotoxicity against the human normal embryonic kidney cell line (HEK293) with IC_{50} =295.63 µM.

Keywords: Co(II) macrocyclic complexes, anticancer activity, antibacterial studies, antifungal studies, template condensation.

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INTRODUCTION

The chemistry of macrocyclic complexes has occupied a central role in the development of coordination chemistry. A crucial area of research that has implications for many other areas of chemistry and biology is the study of macrocyclic complexes. Inorganic chemists are currently interested in a fascinating field of research called macrocyclic ligand coordination chemistry. In general, macrocycles have the capacity to hold a variety of donor atoms, including oxygen, nitrogen, sulphur, etc. As a result, they are crucial for the complexation of metal ions, the production of ammonium and primary alkylammonium salts, as well as for selective ion separation and detection [1-4]. Due to their importance in understanding molecular processes happening in biochemistry [5], material science, catalysis [6], encapsulation, activation, transport [7], separation phenomena, and hydrometallurgy [8], macrocyclic chemistry has gained increased attention. Because of their numerous uses in biological processes including photosynthesis and dioxygen transport, as well as their catalytic capabilities [9], metal extractants, and radiotherapeutic agents [10], macrocyclic complexes are significant. The hexaazamacrocyclic complexes act as catalysts in the electrochemical reduction of carbon dioxide [11]. The biological properties of transition metal macrocyclic complexes, such as their ability to be anti-fertile, anti-tumor, anti-bacterial, anti-fungal [12–15], anti-diabetic, DNA binding, and DNA cleavage have attracted a lot of attention [16].

Because of the challenges associated with diagnosing them and the issue of medication resistance, bacteria and fungus are a hazard in the twenty-first century. Numerous antibacterial and antifungal substances have been discovered and manufactured over the past few decades, although fluconazole, tetracycline, amphotericin-b, and penicillin are still the most commonly used medicines today [17–19].

In numerous enzymes and metalloproteinases, the cobalt metal ion plays a crucial part in the electron transfer reaction that occurs in diverse biological systems. The antimicrobial and anticancer activities of Co(II) macrocyclic complexes is also reported very recently [20]. Additionally, research has been done on the in vitro anticancer efficacy against human breast cancer and human hepatocarcinoma cell lines [21–23]. Due to their extensive use in biology, cobalt complexes have gained significant significance.

In this work, we describe the synthesis and characterization of novel cobalt macrocycles. In order to comprehend the medicinal applications of these metal complexes, an assessment of their antimicrobial and cytotoxic properties has also been carried out.

EXPERIMENTAL

Materials

All chemicals (reagent grade) and solvents (analytical grade) were distilled over appropriate drying agents immediately prior to use. Benzil, 4-chloro-1,2-phenylenediamine, 4-bromo 1,2 - phenylenediamine, 4-nitro 1,2-phenylenediamine, 1,2-diaminotoluene, 1,2- diaminopropane, ethylenediamine and $CoCl_2 \cdot 6H_2O$ were procured from Sigma-Aldrich. The organic solvents as absolute ethyl alcohol, methanol, DMSO, and THF were purified by the recommended methods. ECS 400 MHz (JEOL) spectrometer at MNIT, Jaipur was used to record ^{1}H-NMR spectra (DMSO-d6 solutions) using TMS as the internal standard. IR spectra of the ligand and its cobalt complexes were recorded at FT-IR spectrum (perkin-Elmer) by using the KBr pellet technique. Mass spectra were recorded on Xevo G2-S Q Tof (waters, USA) mass spectrometer at MNIT, Jaipur. UV spectra were recorded on Shimadzu UV3600 UV-VIS-NIR Spectrophotometer at Manipal University, Jaipur.

Methods

Synthesis of ligand (bis(benzil)-4-bromo-1,2- phenylenediamine (LH)

The ligand was synthesized by condensing the EtOH solutions (50 mL) of 4-bromo- 1,2-phenylenediamine (10 mmol, 1.87g) and benzil (20 mmol, 4.20g) in 1:2 molar ratio. The reaction mixture was heated under reflux for 5-7 hours on a ratio head. The resulting mixture was cooled and left in a desiccator overnight to allow the excess solvent to evaporate. The same solvent was used to recrystallize the coloured crystalline products, and the crystals were dried in vacuum. Scheme-1 illustrates the ligand synthesis process.

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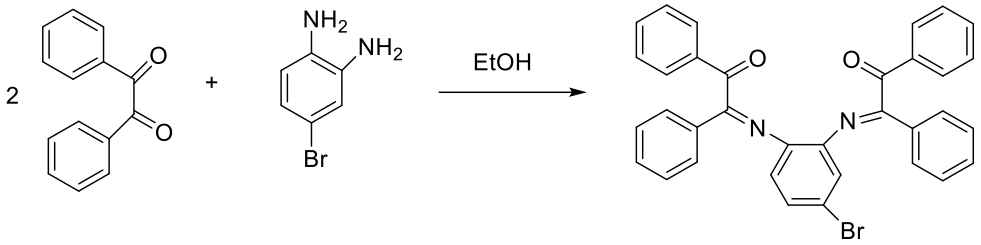


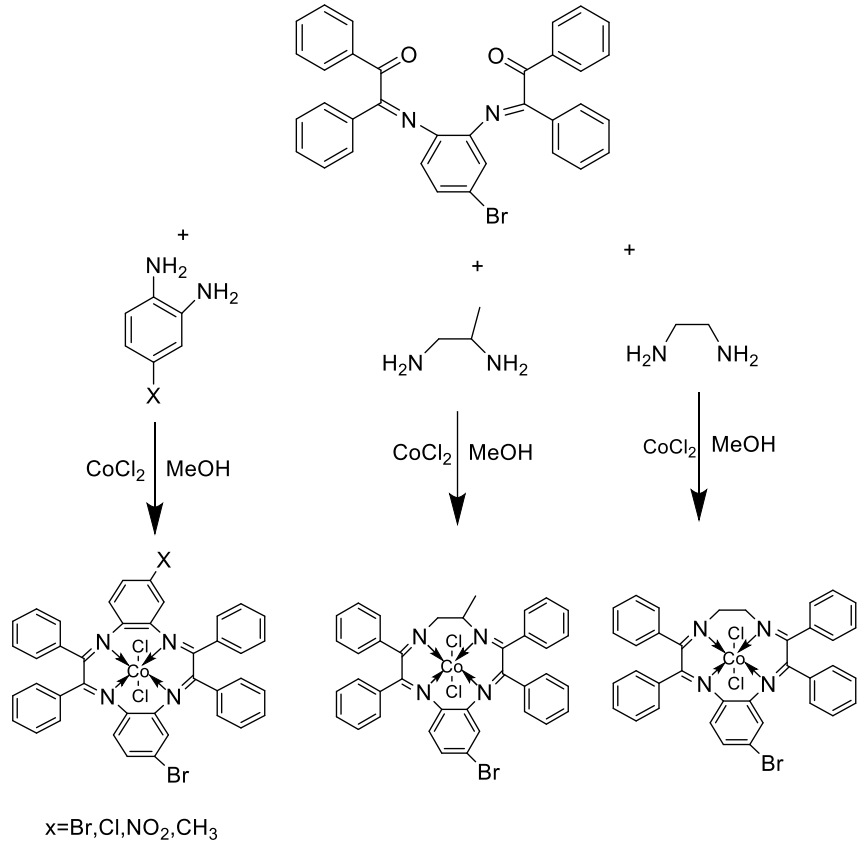
Scheme 1: Synthetic route for the preparation of ligand.

Synthesis of the Co (II) Macrocyclic Complexes

Template condensation of the ligand bis(benzil)-4-bromo- 1,2-phenylenediamine (LH) with various diamine in the presence of $CoCl_2 \cdot 6H_2O$ was done in a ambient condition Scheme 2.

The Co(II) macrocycles were created via the template condensation of ligand (bis(benzil)-4-bromo-1,2- phenylenediamine (LH) with different diamines in the presence of $CoCl_2.6H_2O$ in unimolar (1:1:1) ratio in a methanolic medium. A weighed amount of the ligand LH (5 mmol, 2.85g) was placed in a 100 mL round bottom flask and dissolved in methanol. The solution of the ligand was mixed with the methanolic solution of 4-bromo 1,2-phenylenediamine (5 mmol 0.935g), 4-chloro 1,2-phenylenediamine (5mmol 0.71g), 4- nitro 1,2-phenylenediamine (5mmol 0.765g), 1,2-diaminotoluene (5mmol 0.61g), 1,2-diaminopropane (5mmol 0.37g) and ethylenediamine (5mmol 0.30g) respectively in the presence of $CoCl_2.6H_2O$. The reaction mixture was heated under reflex for about 8-9 h and then concentrated to half its volume and maintained in a desiccator at room temperature. The metal complexes that were produced as solids were washed with methanol and vacuum-dried.

All of the products were recrystallized from a methanol and benzene solution with a molar ratio of 1:1. The complexes' purity was examined using thin layer chromatography (TLC).



Scheme 2: Synthetic route of Co(II) macrocycles

Antibacterial Assay

The antibacterial activity of the newly synthesized compounds was evaluated by a standard microbial technique which is the Agar Well Diffusion method [24]. For the in-vitro antibacterial assay two bacteria E. coli (ATCC25922), and B. subtilis (ATCC6633) were used. The synthesized compounds were diluted by using 10% di-methyl sulphoxide (DMSO) and accordingly 3 distinct concentrations (30 mg/L, 60 mg/L, and 90 mg/L) of all compounds were prepared. Bacteria under study were spreader-inoculated onto clean Petri dishes containing the nutrient agar (NA) medium, and the dishes were left to stand for 30 min. Each agar plate had four 6 mm-diameter wells created inside it. Different concentration of the compound and standard drug (30µL) was poured into the prearranged well of the agar plate. The plates were then incubated for 24h at 37 °C. The compound's antibacterial spectrum was calculated using the inhibition zone (IZ) around each well. The diameters of the inhibition zones created by the various chemical samples were compared to those formed by the common antibiotic Ciprofloxacin (30mg/L, 60mg/L, and 90mg/L), and activity index (AI) was calculated.

Antifungal Assay by Well Diffusion method

The antifungal activity of the standard fungicide **(**Ketoconazole), ligand, and complexes were evaluated using a modified agar well diffusion method [25] for their impact on the development of microbial cultures and investigated for their interactions with *Aspergillus niger* and *Penicillium chrysogenum*. The different sample LH and its metal complexes of three distinct concentration (30mg/L, 60mg/L and 90mg/L) were prepared in 10% Dimethylsulfoxide (DMSO). The fungal strains (7 days old) were inoculated onto petri plates containing potato dextrose agar (PDA) medium and were suspended separately in saline solution. The plates were kept for drying at room temperature for 15 min. four wells of 6 mm diameter were perforated on the agar plate using cork-borers including a control well. A different sample of a compound and standard drug (Ketoconazole) was poured into the wells of preorganized petri plates. The plates were kept for incubation at 28^0C for 96h after this antifungal potential was calculated by computing the diameter of the inhibitory zone (IZ) in mm. All experiments were performed thrice and the mean value was calculated. As a standard control, several concentrations of Ketoconazole (30 mg/L, 60 mg/L, and 90 mg/L) were employed to measure the antifungal activity.
Calculation of activity index:

For the samples, the activity index (AI) was computed as:

$$\text{Activity index (A. I.)} = \frac{\text{Mean of inhibition zone of the sample}}{\text{Inhibition zone obtained for standard antifungal drug}}$$

Anticancer activity

An assessment of cell viability and multiplication of cell numbers was done. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell multiplication assay quantifies the cell division rate. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], DMEM (Dulbecco's modified Eagles medium), trypsin, EDTA and Phosphate Buffered Saline (PBS) were purchased from Sigma Chemicals Co. and Fetal Bovine Serum (FBS) were purchased from Gibco. 25 cm^2 and 75 cm^2 flask and 96 well plates were bought from Eppendorf India.

Maintenance of Cell Line

The cancer cell lines were bought from NCCS, Pune, and kept alive in MEM supplemented with 10% FBS and penicillin/streptomycin (0.5 mL^{-1}), in an environment of 5% CO_2/95% air, at 37^0 C.

Preparation of Test Compound

For MTT assay, each of the test compounds was measured out individually and dissolved in DMSO. Cells were treated with a series of doses ranging from 5 to 100 M in the medium that had been prepared to a final concentration of 1 molar.

MTT Assay

Principle

MTT Assay is a colorimetric assay that determines how much mitochondrial succinate dehydrogenase reduces yellow MTT. The test is based on the presence of a certain number of cells as well as the presumption that dead cells or the products they produce do not decrease tetrazolium. MTT penetrates the cells and travels to the mitochondria, where it is broken down into insoluble formazan crystals that are dark purple in colour. The released solubilized formazan reagent is then detected spectrophotometrically at 570 nm after solubilizing the cells with DMSO [26].

Procedure

The MTT test was used in three separate studies with triplicates of six different doses of the compound to assess the viability of the cells. Cells were trypsinized and the tryphan blue test was used to determine the viability of cells in suspension. Cells were counted by haemocytometer and seeded at the density of 5.0 X 10^3 cell/well in 100 µL media in 96 well plate culture medium and incubated overnight at 37^0C. After incubation, the old media was removed and 100 µL of new media with various test chemical concentrations were added to the corresponding wells in 96 plates [27]. After 48h., fresh medium and MTT solution (0.5 mg mL^{-1}) were added to each well after the drug solution was discarded, and the plates were then incubated at 37°C for 3 hours. Precipitates are created at the conclusion of the incubation period as a result of the cells with metabolically active mitochondria reducing the MTT salt to chromophore formazan crystals. On a microplate reader, the optical density of crystals that had been solubilized in DMSO was determined at 570 nm. the following formula was used to compute the percentage growth inhibition.

$$\% \text{ Inhibition} = \frac{100\ (\text{Control} - \text{Treatment})}{\text{Control}}$$

The IC_{50} value was calculated by utilizing a linear regression equation i.e. y = mx + c Here, y= 50, m and c values were obtained from the viability graph.

RESULTS AND DISCUSSION

IR Spectra

Table 1 lists the ligand and its Co (II) complexes' absorption frequencies and their corresponding assignments. In the spectra of diamines bands due to $\upsilon_{as}(NH_2)$ at 3385 cm^{-1}, $\upsilon_s(NH_2)$ at 3255 cm^{-1} were observed while ligand exhibited a strong band for υ(C=O) in the region 1670 cm^{-1}. The absence of these bands in the spectra of Co(II) macrocycles and the presence of an absorption band near 1585–1640 cm^{-1} confirms the formation of macrocyclic framework. The presence of bands at 1460–1490 cm^{-1} and 1360–1395 cm^{-1}, which are indicative of benzil moieties and can be attributed to $\nu_{asym}C_6H_5$ and $\nu_{sym}C_6H_5$, respectively, further supports this claim. The emergence of a new band with a medium intensity in the 425–440 cm^{-1} region, attributable to υ(Co-N), is a significant characteristic and offers compelling proof that the imine nitrogen is coordinated to the metal ion.

Table 1: IR (cm^{-1}) and ^{1}H NMR (§ ppm) spectral data of the ligand and its matel complexes.

Entry	Compound	IR Spectra data(cm^{-1}) υ(C=O)	IR Spectra data(cm^{-1}) υ(C=N)	IR Spectra data(cm^{-1}) υ(Co←N)	^{1}H NMR Spectra data (ppm)Aromatic proton(m)
1	$[C_{40}H_{26}Br_2Cl_2CoN_4]$	-	1620	421	-
2	$[C_{40}H_{26}BrCl_3CoN_4]$	-	1635	445	-
3	$[C_{40}H_{26}BrCl_2CoN_5O_2]$	-	1635	430	-
4	$[C_{41}H_{29}BrCl_2CoN_4]$	-	1640	425	-
5	$[C_{37}H_{29}BrCl_2CoN_4]$	-	1610	420	-
6	$[C_{36}H_{27}BrCl_2CoN_4]$	-	1587	428	-
7	$[C_{34}H_{23}BrN_4O_2]$	1666	1585	-	7.24-8.21

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^{1}H-NMR Spectra

Proton NMR Spectral data was collected in DMSO-d6 providing an additional support for the formation of precursor. The signals arising due to primary amino protons of diamines were absent in the spectra of LH. The spectra of LH exhibit resonance signals at 7.24–8.21 ppm, appearing as multiplets which may be assigned to aromatic protons.

Mass spectra

In coordination chemistry, mass spectroscopy which is mostly utilized in the investigation of complexes has become a potent tool for structural characterization. Peaks of significant intensity were recorded at m/z values 572.47 for $[C_{34}H_{23}BrN_2O_2+1]^+$, 853.32 for $[C_{40}H_{26}Br_2Cl_2CoN_4+1]^+$, 808.86 for $[C_{40}H_{26}BrCl_3CoN_4+1]^+$, (Figure 1) 819.42 for $[C_{40}H_{26}BrCl_2CoN_5O_2+1]^+$, 788.45 for $[C_{41}H_{29}BrCl_2CoN_4+1]^+$, 740.40 for $[C_{37}H_{29}BrCl_2CoN_4+1]^+$ 726.37 for $[C_{36}H_{27}BrCl_2CoN_4+1]$. These observations support the cobalt (II) complex's mononuclear nature and are in good agreement with the complex's corresponding predicted molecular mass.

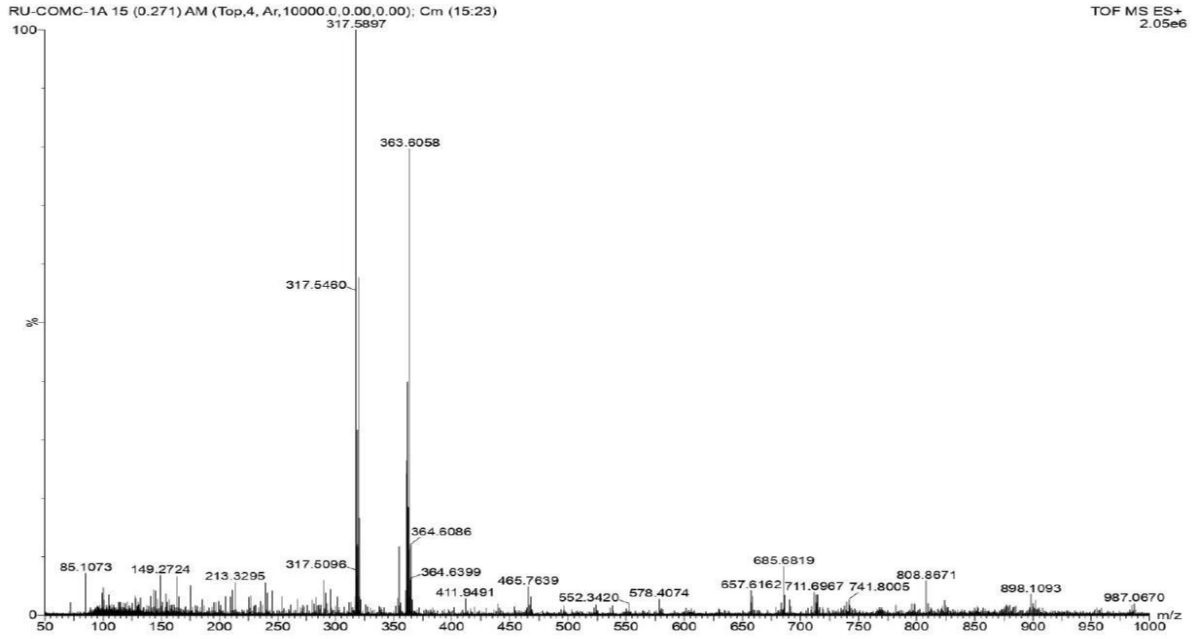

Figure 1: Mass spectrum of the $[C_{40}H_{26}BrCl_3CoN_4+1]^+$ complex

Electronic spectra

The electronic absorption spectra of LH and Co(II) macrocycles were captured in distilled DMSO. The absorption maxima at 275–345 nm in the spectra of the ligand is due to $\pi \rightarrow \pi^*$ electronic transitions which remains almost at the same position in the spectra of the Co(II) macrocycles. The spectra exhibit a broad band at ~395 nm in the ligand that can be assigned to the azomethine group's n-π^* transitions, which experience a blue shift as a result of chelation in the complexes' spectra [28]. This shift indicates that nitrogen is coordinated with the Co(II) metal ion. The electronic absorption spectra of the Cobalt (II) macrocycles show three d–d bands in the range of 9000–21,800 cm^{-1} which are allowed by spin selection rule and corresponds to $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ transitions respectively, putting forth an octahedral geometry for Co (II) [29]. Table 2 lists the many ligand field parameters that have been determined, including Dq, B, and β.

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Table 2: Electronic spectral data of Co(II) complexes.

Compound	Transition	Spectral band (cm^{-1})	Dq	B	β	ν2/ν1	β%
$[C_{40}H_{26}Br_2Cl_2CoN_4]$	$^4T_{1g}(F)\rightarrow{}^4T_{2g}(F)$ (υ_1) $^4T_{1g}(F)\rightarrow{}^4A_{2g}(F)$ (υ_2) $^4T_{1g}(F)\rightarrow{}^4T_{1g}(P)$ (υ_3)	9333 15384 21052	605.1	562.4	0.579	1.64	*42.1*
$[C_{40}H_{26}BrCl_3CoN_4]$	$^4T_{1g}(F)\rightarrow{}^4T_{2g}(F)$ (υ_1) $^4T_{1g}(F)\rightarrow{}^4A_{2g}(F)$ (υ_2) $^4T_{1g}(F)\rightarrow{}^4T_{1g}(P)$ (υ_3)	9420 15157 21739	573.7	575.7	0.592	1.60	40.8
$[C_{40}H_{26}BrCl_2CoN_5O_2]$	$^4T_{1g}(F)\rightarrow{}^4T_{2g}(F)$ (υ_1) $^4T_{1g}(F)\rightarrow{}^4A_{2g}(F)$ (υ_2) $^4T_{1g}(F)\rightarrow{}^4T_{1g}(P)$ (υ_3)	9100 15035 20833	593.5	571.2	0.588	1.65	41.2
$[C_{41}H_{29}BrCl_2CoN_4]$	$^4T_{1g}(F)\rightarrow{}^4T_{2g}(F)$ (υ_1) $^4T_{1g}(F)\rightarrow{}^4A_{2g}(F)$ (υ_2) $^4T_{1g}(F)\rightarrow{}^4T_{1g}(P)$ (υ_3)	9005 15267 20010	626.2	550.6	0.567	1.69	43.3
$[C_{37}H_{29}BrCl_2CoN_4]$	$^4T_{1g}(F)\rightarrow{}^4T_{2g}(F)$ (υ_1) $^4T_{1g}(F)\rightarrow{}^4A_{2g}(F)$ (υ_2) $^4T_{1g}(F)\rightarrow{}^4T_{1g}(P)$ (υ_3)	9415 15416 21600	600.1	584.7	0.602	1.63	39.2
$[C_{36}H_{27}BrCl_2CoN_4]$	$^4T_{1g}(F)\rightarrow{}^4T_{2g}(F)$ (υ_1) $^4T_{1g}(F)\rightarrow{}^4A_{2g}(F)$ (υ_2) $^4T_{1g}(F)\rightarrow{}^4T_{1g}(P)$ (υ_3)	9365 15542 21241	617.7	579.2	0.596	1.65	40.4

Biological assay

All of the compounds had their antibacterial abilities assessed, and MIC- the lowest concentration at which microbe growth is inhibited—was noted. The microorganisms used were E. coli (ATCC25922), B. subtilis (ATCC6633), *A. niger* and *P. chrysogenum*.

Minimum Inhibitory Concentration

A given volume of the substance was diluted to three different concentrations which are 30 mg/L, 60 mg/L and 90 mg/L using the Macro-broth dilution procedure to calculate the minimum inhibitory concentration (MIC) of the samples [30]. Using a sterile cork borer (6mm), wells were made on the agar surface and labelled suitably. Various dilutions of the sample prepaered were used to fill up the wells with the help of a Pasteur pipette and then the plates are subjected to incubation at 28^0C (24 hours for bacteria and 28^0C for 96 hours for fungi). The Minimum Inhibitory Concentration (MIC) was defined as the lowest sample concentration at which the growth of the test organism is inhibited (MIC) [31-32].

The ligand LH and its cobalt (II) macrocycles were tested against two bacterial strains, *Escherichia coli* and *Bacillus subtilis,* and two fungal strains, *Aspergillus niger* and *Penicillium chrysogenum to* assess their antimicrobial potential. The results are depicted in Figure 2 and 3.

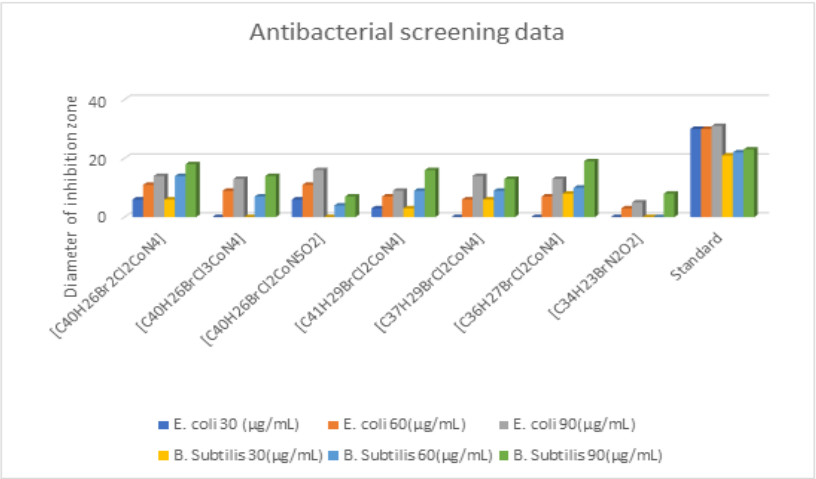

Figure 2: Antibacterial activity screening data for the ligand and its complexes.

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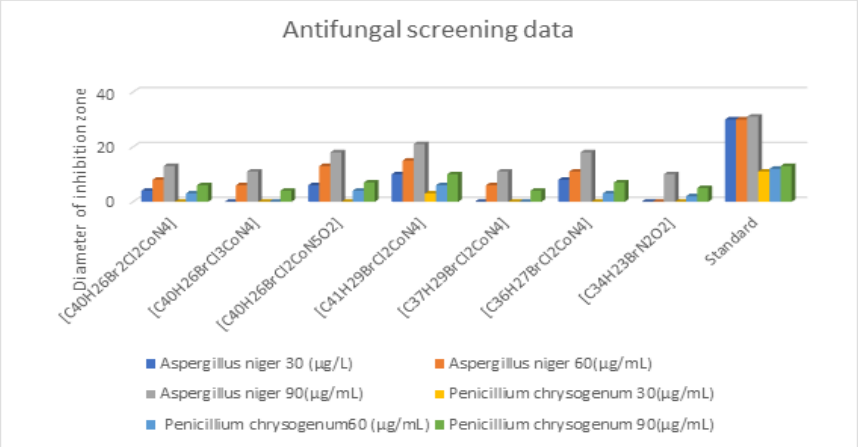

Figure 3: Antifungal activity screening data for the ligand and its complexes.

All bacterial and fungal strains were found to be sensitive to the ligand and its respective Co (II) derivatives. According to the results of the antimicrobial screening, metal complexes are more effective antibacterial agents than the free ligand. The antifungal and antibacterial activities rise with rising concentration. Thus, it is clear that concentration is essential for increasing the level of inhibition. Tweedy's chelation theory, according to which partial sharing of the core metal atom's positive charge with the ligand during chelation decreases the polarity of the metal which favours the permeation of the complexes through the lipid layer of the cell membrane. This would imply that chelation might make it easier for a complex to pass through a cell membrane. Table 3 displays the MIC values for the ligand and its cobalt (II) complexes. The activity of Co(II) complexes against all bacterial, and fungal, strains is moderate to good. In order to determine these complexes' potential as future antimicrobial agents, more research needs to be done on them. Compared to bacterial strains, metal complexes are more effective against fungi.

Table 3: MIC of ligand and its macrocyclic metal complexes against fungi *A. niger and P. chrysogenum* antibacterial *E. coli* and *B. subtilis*

Compound	***A. Niger*** **(µg/mL)**	***P. chrysogenum*** **(µg/mL)**	***E. coli*** **(µg/mL)**	***B. subtilis*** **(µg/mL)**
$[C_{40}H_{26}Br_2Cl_2CoN_4]$	30	60	30	30
$[C_{40}H_{26}BrCl_3CoN_4]$	50	90	50	50
$[C_{40}H_{26}BrCl_2CoN_5O_2]$	30	60	20	60
$[C_{41}H_{29}BrCl_2CoN_4]$	30	50	30	30
$[C_{37}H_{29}BrCl_2CoN_4]$	40	90	40	30
$[C_{36}H_{27}BrCl_2CoN_4]$	30	60	40	20
$[C_{34}H_{23}BrN_2O_2]$	80	60	60	80

Cytotoxic activity of complexes

Two cancer cell lines namely breast (MCF-7) and cervical (HeLa) were used to evaluate the antiproliferative effect of synthesized compounds. Moreover, the human embryonic kidney cell line, HEK293 was also used as a reference for normal cells. Cisplatin was used as a standard anticancer agent. The observed IC_{50} value for cisplatin against the HeLa cell line and MCF-7 cell line was 5.62 µM and 7.53 µM, respectively. The median inhibitory concentration (IC_{50}) values and viability graph were determined for each cell line (Table 4) (Figure 4,5,6) compound $[C_{40}H_{26}Br_2Cl_2CoN_4]$ exhibit selective cytotoxicity against cervical cancer line (HeLa) with IC_{50} = 85.88 µM and weak action against breast cancer cells, however, it was safe on HEK293 with IC_{50} = 295.63 µM for the human normal embryonic kidney cell line (Figure 7). The selectivity index (SI) of $[C_{40}H_{26}Br_2Cl_2CoN_4]$ for cervical cancer cells is 3.44, which is higher than the selectivity index (SI) of the reference medication cisplatin [33]. The selectivity index (SI) value indicated the selectivity of the test compounds toward the cancer cells tested. A compound with more than 3 selectivity index value is considered highly selective for a certain cell line [34]. With increasing concentrations of the tested substances, the HeLa cell line and MCF-7 cell line experience a greater

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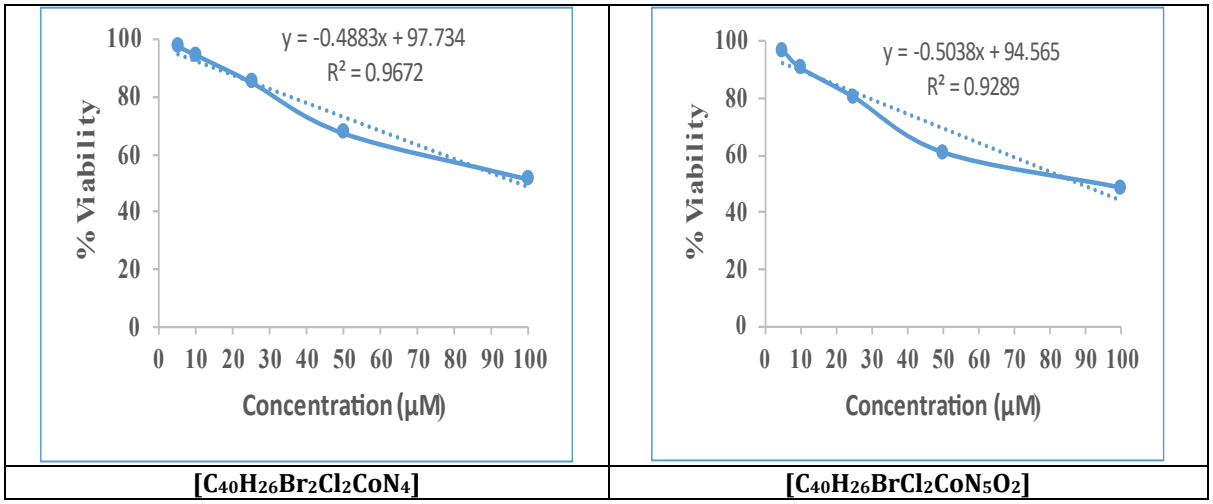
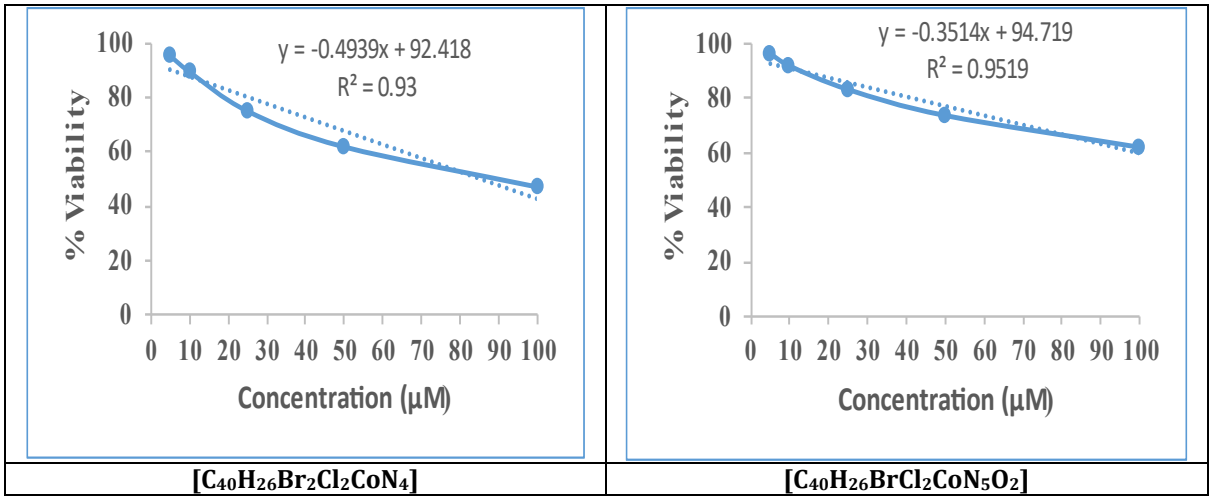
percentage of growth inhibition. The low IC_{50} values of Co (II) complexes may be related to their poor solubility, limited bioavailability, and preferential selectivity towards the specific cell.



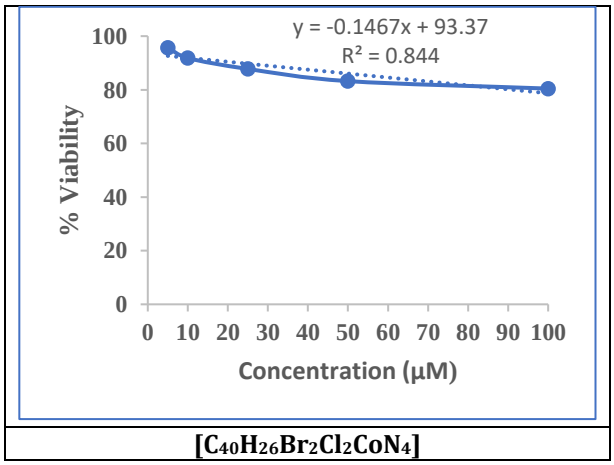
$[C_{40}H_{26}Br_2Cl_2CoN_4]$	$[C_{40}H_{26}BrCl_2CoN_5O_2]$

Figure 4: Viability graph of Co(II) complexes on HeLa cell line



$[C_{40}H_{26}Br_2Cl_2CoN_4]$	$[C_{40}H_{26}BrCl_2CoN_5O_2]$

Figure 5: Viability graph of Co(II) complexes on MCF-7 cell line



$[C_{40}H_{26}Br_2Cl_2CoN_4]$

Figure 6: Viability graph of $[C_{40}H_{26}Br_2Cl_2CoN_4]$ metal complex on HEK293cell line

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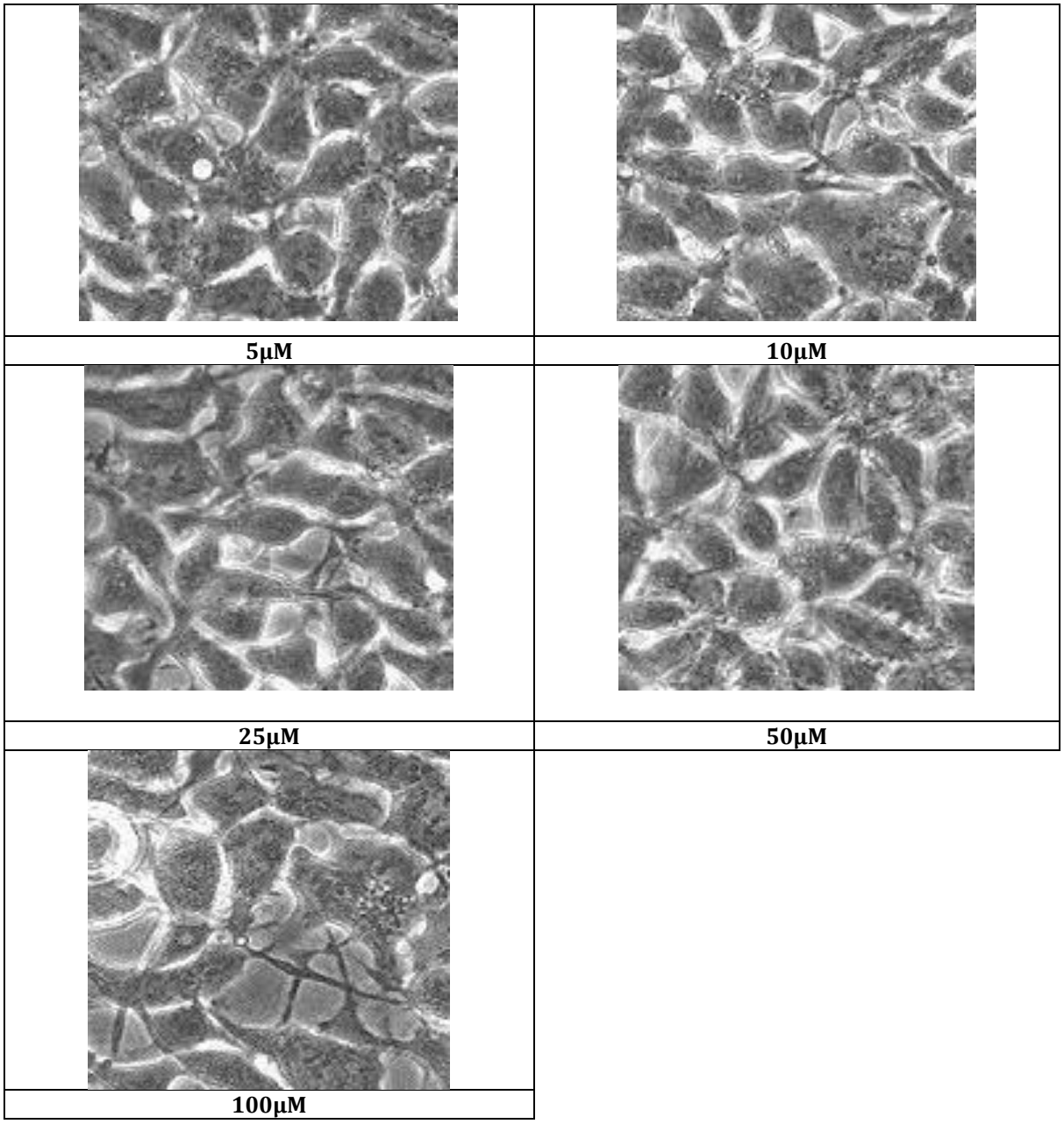

Figure 7: Photomicrographic image of human normal embryonic kidney cell line (HEK293) after treatment with [$C_{40}H_{26}Br_2Cl_2CoN_4$] complex at different concentrations.

Table 4: The IC_{50} value of the ligand Co(II) complexes on the HeLa cell line and MCF-7 cell line

S. No	Complexes	IC_{50} (µM)		
		HeLa	MCF-7	HEK 293
1	[$C_{40}H_{26}Br_2Cl_2CoN_4$]	97.75	85.88	295.63
2	[$C_{40}H_{26}BrCl_2CoN_5O_2$]	88.45	127.25	
3	Cisplatin	5.62	7.53	

CONCLUSION

Synthesis of Co (II) macrocyclic complexes their characterisation, and biological activity have been the main subjects of this study. IR, UV, ^{1}H-NMR, and EI mass spectroscopy were used to physically

characterise the ligand and its compounds. On the basis of spectroscopic data an octahedral geometry has been proposed for the synthesized macrocyclic complexes. The results revealed the formation of monomeric macrocyclic metal complexes. The ligand and its complexes were tested against various bacterial and fungal species. Chelation theory has been used to explain the increased activity of the macrocyclic complexes compared to the parent ligand. Cytotoxic properties of ligand and metal complexes were screened against HeLa (cervical cancer) and MCF-7 (human breast cancer) cell lines. In addition, the complex [$C_{40}H_{26}Br_2Cl_2CoN_4$] was also screened against normal cell line (HEK293) to determine the selectivity index SI. The result showed a higher value of SI (>3) which suggest that the compound is sensitive towards cancer cell line and not toxic to normal cells.

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