Letters in Applied NanoBioScience
Platinum Open Access Journal (ISSN: 2284-6808)

Article
Volume 9, Issue 4, 2020, 1655 - 1672
https://doi.org/10.33263/LIANBS94.16551672

Novel Benzimidazole Derived Imine-Based Ligand and its Co(III), Ni(II), Cu(II) and Pt(II) Complexes: Chemical Synthesis, Structure, Antimicrobial, DNA Interaction Studies and Nuclease Activity

Prakasha Gavisiddegowda [1], Shiva Prasad Kollur [2], Syed ibrahim [1], Hosakere Doddarevanna Revanasiddappa [1]

[1] Department of Studies in Chemistry, Manasagangotri, University of Mysore, Mysore, Karnataka – 570 006, India
[2] Department of Sciences, Amrita School of Arts and Sciences, Amrita Vishwa Vidyapeetham, Mysuru Campus, Mysuru, Karnataka – 570 026, India
* Correspondence: hdrevanasiddappa@yahoo.com;

Scopus Author ID 7004879365
Received: 11.07.2020; Revised: 12.08.2020; Accepted: 14.08.2020; Published: 19.08.2020

Abstract: Herein, we report the synthesis of a Schiff base ligand, 2-((E)-((1*H*-benzo[d]imidazol-2-yl)methylimino)methyl)-4,6-dichlorophenol (BMDC) and its Co(III), Ni(II), Cu(II) and Pt(II) coordinated compounds (henceforth named as M1, M2, M3, and M4, respectively). The synthesized compounds were subjected to various analytical and physical characterization techniques to confirm their molecular structures. Further, the DNA binding and cleavage propensity of the as-prepared metal complexes were evaluated using calf thymus DNA (CT-DNA) and supercoiled plasmid DNA (pBR322). The DNA interaction results revealed that the tested metal complexes bind with DNA through the covalent and non-covalent mode of interaction, which is investigated using absorption and fluorescence spectral studies followed by viscosity measurement. The scission of supercoiled plasmid DNA by the metal complexes suggested the potentiality of the molecules toward the cleavage of pBR322 DNA. Furthermore, the compounds were screened for *in vitro* antiproliferative activity, tested against the various cell lines such as A549, EAC, SIHA, and NIH3T3. Results revealed that compound M4 exhibited marked anti-proliferative activity against EAC cell line with a significant IC_{50} value of 10 µm compared to its parent ligand, BMDC, and other metal transition complexes under study. In addition, various hematological parameters (alkaline phosphate, creatinine, urea, RBC, and WBC) were studied, and significant results are obtained from the experiments.

Keywords: Benzimidazole; Imine-based ligand; Spectral techniques; DNA interaction; Anti-proliferative activity.

1. Introduction

Even though a lot of research has been made on a benzimidazole moiety, still there is the everlasting challenge of making effective, clinically potent, synthetically flexible and novel series of benzimidazole molecules in the field of bioinorganic and medicinal chemistry [1]. Benzimidazole metal complexes catch much more attention due to vivid biological activities such as antibacterial, antifungal, anti-inflammatory, and anti-cancer activities [2]. In the line of discovering new cytotoxic drugs at the cellular level, a primary step to expose the metallodrugs to interact with DNA which is the ultimate cellular target of every chemotherapeutics [3].

Metal complexes play an important role in the development of anti-cancer drugs due to their high stability in various conditions and the ability to interact with biomolecules in the human body. The establishment of cytotoxicity of cisplatin (cis-$Pt(NH_3)_2)Cl_2$) moiety by Rosenberg in late 1960 is the strong evidence for the metal complexes have been employed in antitumor drugs discovery [4]. Copper is less toxic than other non-essential metal ions and which is an essential endogenously biocompatible metal ion obtained in the living system. In various pharmacy studies, people are preferred to prepare copper complexes containing bio-relevant ligand due to the potent binding and cleavage activity with the nucleic acids [5]. Cobalt indirectly exhibited the big role in the DNA synthesis, and it is an essential biological element in the cobalt-dependent proteins [6]. The complexes containing Co(III) ion was widely popular in coordination chemistry and biomedical field due to their therapeutic c behavior [7]. The knowledge of the role of nickel metal in the characterization of urease as a nickel enzyme since 1975 in bioinorganic chemistry has been rapidly investigated [8]. The binding interaction between the nickel complexes and the DNA found to mainly depend on the structural futures of the ligand attributed intercalative nature [9].

The various interaction of the complex with DNA gives us valuable information in controlling cell apoptosis and cell division which can be confirmed by calculating binding constant through electronic absorption studies, fluorescence quenching method and Viscosity measurements [10]. Further, the resultant complexes can be exploited to DNA cleavage studies by electrophoretic gel method.

In the present work, an imine-based ligand, BMDC was synthesized by reacting 2-(Aminomethyl) benzimidazoledihydrochloride with 5,3-dichloro-2-hydroxybenzaldehyde in 1:1 ratio in methanol. This was further used to prepare transition metal complexes in different stoichiometric ratios. The structure of all the compounds was evaluated using analytical and physicochemical techniques. In order to know the biological potency of the above-prepared molecules, DNA binding, nuclease interaction, and anti-proliferative activities were evaluated. Following sections detail the preparation, characterization, and biological activities of the molecules under study.

2. Materials and Methods

2.1. Materials and methods.

2-(aminomethyl)benzimidazoledihydrochloride, K_2CO_3, 3,5-dichlorosalicyladehide, methanol, tris-buffer, and Ethidium bromide were purchased from Avra, and Sigma Aldrich Limited (India). All the solvents and chemicals were utilized without further purification. HAS and CT-DNA were procured from Merck, Hyderabad, India, and stored at 4 °C and used for the experiments when required. Triple distilled water was used to prepare a buffer solution for DNA and HAS interaction studies.

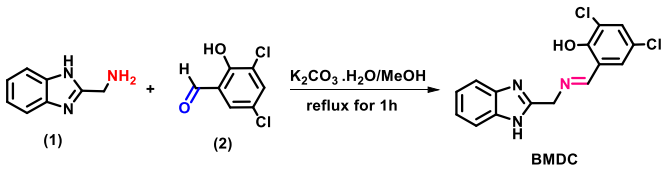
Perkin-Elmer CHN 2400 elemental analyzer was used to determine the contents of C, H, and N. The FT-IR spectra of ligand and its metal complexes were recorded in range 4000 cm^{-1} to 400 cm^{-1} with a Bruker IFS66V in KBr pallets and all the complexes are recorded in polyethylene medium (Nujol-mull method). Electronic excitation of the transition metal complexes was measured in the range of 200 nm to 850 nm using DMSO as a solvent with Perkin-Elmer Lambda 35 spectrometer. Elico model conductivity meter was used to perform the conductance of prepared complexes in DMSO (10^{-3}) solution at room temperature. ^1H and ^{13}C-NMR spectra were recorded on Brucker 400 MHz instrument using DMSO-d_6 as a solvent,

and the signals were assigned with reference to TMS as an internal standard. Polmont instrument confirms the melting point of synthesized compounds. Molecular mass investigation of the compounds was done through electron spray ionization (ESI) method on a mass spectrometer (Waters, Germany). The magnetic moment of the resultant complexes resolved on Guoy's balance using $Hg[Co(CN)_4]$ as standard material. TGA-DTG investigation of all the synthesized metal complexes was measured on NETZCH-ST 409PC thermal analyzer under dynamic nitrogen atmospheric condition with a heating rate of 10 °C/min. JES-FA200 ESR spectrometer was used to identify the magnetically active metal center in prepared complexes at liquid nitrogen temperature. The combining capacity of CT-DNA with complexes was performed by using a UV-Visible spectrophotometer. Combining capacity was further confirmed by fluorescence measurement on RF-5301P spectrofluorimeter, and cleavage activity was performed on agarose gel electrophoretic experiment using supercoiled pBR322 plasmid DNA.

2.2. Chemical synthesis.

2.2.1. Preparation of 2-((E)-((1H-benzo[d]imidazol-2-yl)methylimino)methyl)-4,6-dichlorophenol (BMDC).

The ligand was synthesized (Scheme 1) by the neutralization of 2-(aminomethyl) benzimidazoledihydrochloride (1) (0.1 g, 0.4543 mmol) with aqueous solution of potassium carbonate (0.0753 g, 0.5451 mmol). The resultant solution was then mixed with a methanolic solution of 5,3-dichloro-2-hydroxybenzaldehyde (2) (0.0630g, 0.4543 mmol). The above reaction mixture was stirred at room temperature using magnetic stirred for 2 h. During the reaction, yellow precipitation was obtained, which is then filtered and washed with distilled water followed by petroleum ether [11]. The completion of the reaction was monitored by TLC (pre-coated silica gel plates, Merck) using chloroform and methanol as a solvent system in the ratio (9:1). The prepared ligand, BMDC is insoluble in water and non-polar solvents but soluble in many polar solvents (methanol, DMSO, chloroform, etc.). Elemental analysis ($C_{15}H_{10}Cl_2N_3O$): Found (%) C, 62.95; H, 4.36, N, 14.65. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm) δ = 5.06 (s, 2H, CH_2), 7.14 (d, 2H, Ar-H), 7.43-7.506.(d, 2H, Ar-H), 7.57(s, 1H, Ar-H), 7.62 (s, 1H, Ar-H) 8.76 (s, 1H, N=CH), 12.51 (s, 1H, Ar-OH), 14.6 (s, 1H, NH in imidazole ring) (Figs. 1-3).



Scheme 1. Synthesis of (2-((E)-((1H-benzo[d]imidazol-2-yl)methylimino)methyl)-4,6-dichlorophenol (BMDC).

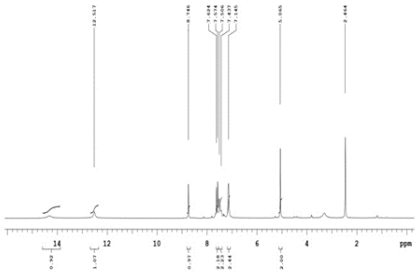


Figure 1. ^{1}H NMR spectra of ligand BMDC.

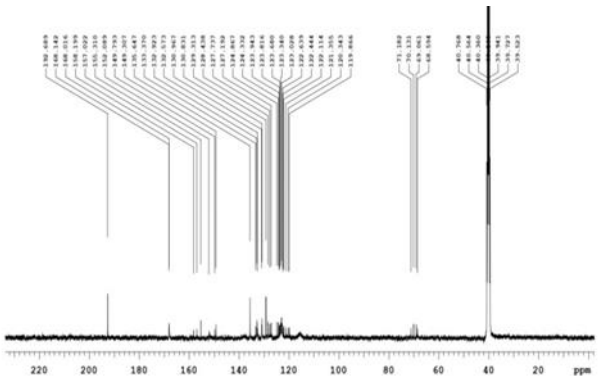


Figure 2. ^{13}C NMR spectra of ligand BMDC.

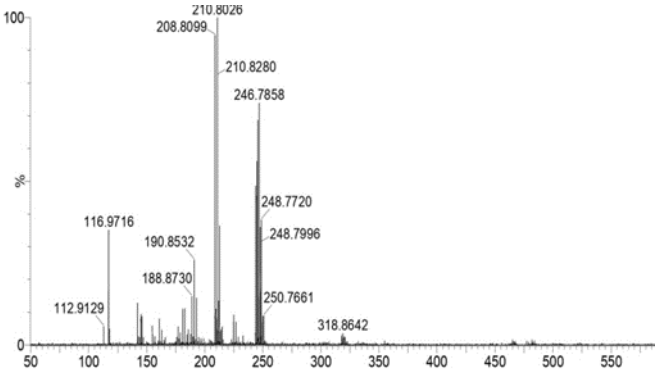


Figure 3. Mass spectra of ligand BMDC.

2.2.2. Synthesis of Co(III) and Ni(II) complexes in the ratio 1:2 (M1 & M2).

The imine based cobalt and nickel metal complexes were synthesized by adding hot methanolic solution of the metal chlorides ($CoCl_2$.$6H_2O$ and $NiCl_2{\cdot}2H_2O$) into a methanolic solution of ligand (BMDC). The resultant solutions are refluxed for 6 to 7 h on a water bath to get required complexes of Co(III) and Ni(II) as brown and red color precipitate, respectively.

2.2.3. Synthesis of Cu(II) and Pt(II) complexes in the ratio 1:1 (M3 and M4).

The imine based copper and platinum metal complexes were synthesized by adding a hot metabolic solution of metal chlorides ($CuCl_2.2H_2O$ and $PtCl_2{\cdot}2H_2O$) into a methanolic solution of ligand (BMDC). The resultant solution was refluxed for 6 to7 h on a water bath to get required complexes of Cu(II) and Pt(II) as green and orange color precipitates, respectively. The proposed structures are shown in Fig. 4.

2.3. Biological assays.

2.3.1. Antimicrobial activity. Six bacterial strains were selected from the present investigation.

Three from Gram-positive bacteria viz., *B. Subtilis, S. aureus, L. Monocytogenes* and other three from Gram-negative bacteria viz., *E. Coli*, *P. aerogenes* and *V. cholera.* The bacterial stock cultures were incubated at 37 °C for 24 h on nutrient agar and kept in the refrigerator at 5 °C. To perform the antibacterial activity of the ligand and its complexes, the antibacterial agar well diffusion assay was study in accordance with the method with slight modification [12]. The dimethyl sulfoxide (5% DMSO, v/v) as a solvent was used to prepare the ligand and its transition metal complexes at 1 mg/mL. The highest dilution (lowest

concentration) required to quench the bacteria growth was commonly known as minimum inhibitory concentration (MIC). The serial plate dilution was used to screen the newly synthesized compounds.

2.3.2. DNA binding studies.

It is necessary to explore the small molecules interact with DNA because due to the fact that DNA is the primary pharmacological goal of antitumor drugs. Particularly, well-tailored ligand frameworks of metal complexes are fit for the molecular target site in the surface future and the shape that complements. Hence, clearly understood the mechanism by which drugs binding with nucleic acid and their link with significant biological effects have been a serious focus in medicinal chemistry. The binding mode of interaction between the resultant transition metal complexes and CT-DNA determines its capacity as a chemotherapeutic agent. Thus, the interaction of the synthesized metal complexes with DNA was evaluated presently via electronic absorption titration, fluorescence quenching experiment, and viscosity measurement method.

2.3.2.1. Electronic absorption studies.

The stock solutions of complexes were prepared in DMSO at 2.2×10^{-2} $molL^{-1}$. The solution was diluted up to the required concentration using Tris-HCl buffer solution at pH 7.2. Electronic absorption spectra were performed at room temperature by a constant concentration of metal complexes at $3.0 \times 10^{-5} molL^{-1}$ with a concentration of DNA ranging from 0 to 2.5×10^{-5} $molL^{-1}$. Electronic absorption spectra were recorded at 230-550 nm, and Tris-HCl buffer was used as blank [13].

2.3.2.2. Fluorescence quenching experiment.

The concentration of DNA and Ethidium bromide was fixed at 5.0×10^{-5} $molL^{-1}$ and alter the concentration of complexes from 0 to 2.5×10^{-5} $molL^{-1}$ were used to perform the fluorescence quenching experiment [14]. All the solutions were diluted by using Tris-HCl buffer (pH 7.2). The fluorescence spectra were measured at the excitation wavelength (λ_{max}) at 525 nm and emission wavelength (λ_{max}) between 535 to 680 nm.

2.3.2.3. Viscosity measurement.

Complexes were added into DNA ($3.65\times10^{-4} molL^{-1}$) with microsyringe, were used to administrate the viscosity measurement. Maintain the samples' concentration range of 0 t0 $2.25\times10^{-4} molL^{-1}$. The equation $\eta = (t\text{-}t_0)/ t_0$ was used to evaluate the relative viscosities η, the DNA solution flow time through the capillary in the presence and absence of complexes represented by t and t_0. Viscosities of the samples were evaluated by the average value of three replicated measurements [15]. Data were presented as the ratio of the concentration of the compound to the DNA concentration versus $(\eta/ \eta_0)^{1/3}$, the η and η_0 are the concentration of DNA with and without compounds.

2.3.3. Cleavage activity with pBR322 plasmid DNA.

As is known, metal complexes have a great tendency to binding with the DNA double-helical structure, thus denature the structure of DNA. This special ability of metal complexes

promotes the focus to investigate carcinoma, where the abnormal duplication of healthy cells spreading may stop by the affected DNA cells denaturing. In this manner, the DNA cleavage property of Pt(II) complex was administrated via the concentration-dependent cleavage of DNA agarose gel electrophoretic method in the absence of reductant supercoiled pBR322 plasmid DNA as a target [16]. The compound was placed at 32 °C for about 5 h with the concentration 10 µM in aqueous buffer solution (50mM Tris-HCl/50 mM NaCl, pH 7.2) with the addition of hydrogen peroxide. , The product of the DNA cleavage, was subjected to electrophoresis, and photograph of the band were observed under UV light.

2.3.4 Pharmacology.

The *in vitro* cytotoxicity and anti-proliferative activity of the synthesized ligand and its complexes M1-M4 were initially screened against various cell lines such as A549, EAC, SIHA and NIH3T3. The complex M4 exhibited significant IC_{50} value for the Ehrlich ascites carcinoma (EAC) cell line; therefore, it was further investigated for its hematological serum profile parameters activity using standard producer [17].

2.3.4.1 Cell culture and in vitro treatment.

The A549, EAC, SIHA and NIH3T3 were grown in DMEM medium, supplemented with 10% heat-inactivated Foetal Bovine Serum (FBS), antibiotic-mycotic and sodium bicarbonate (0.37%) and placed at 37 °C in a 5% carbon dioxide and atmospheric air 95%, with 98% humidity. The cells were treated with varying concentrations of synthesized BMDC ligand, and its complexes (0-50 µM in DMSO) for 48 h and cytotoxic efficacy of BMDC ligand and its complexes was studied by MTT assay as described previously and IC_{50} values were calculated [18]. 5-fluorouracil was used as a positive control for all the experiments. The experiment was repeated at list three independent times and analyzed.

2.3.4.2 MTT assay.

The MTT assay was first screened to investigate the extent of proliferation against various cell lines such as A549, EAC, SIHA, and NIH3T3. The cells were subjected with BMDC ligand, its complexes, and 5-fluorouracil in accordance with the method described previously [19]. The cells treated with compounds and that without were placed for 48 h, followed by the addition of MTT reagent (5 mg/mL) and the color difference due to the proliferating cell was evaluated. The Pt(II) complex concentration resulting in a 50% inhibition in cell viability (IC_{50}) value was analyzed after two days of treatment.

2.3.4.3 Hematological and serum profile parameter.

In order to carry out the investigation of hematological and serum profile parameters viz WBC, RBC, alkaline phosphate, urea, and creatinine from normal mice as well as EAC cell bearing mice, the standard methods cell dilution fluids and hemocytometer were followed. To this investigation, mice were grouped into 10 (n=4), for five groups, tumors were transplanted, and treatments will be initiate immediately after 24 hrs. Group 1 are kept as untreated, Group 2 received a standard drug bleomycin (0.3mg/kg,i.p.) and Group 3-5 were treated by complex M4 at incremental doses of 2.5mg/kg(i.p.), 5.0mg/kg(i.p.)and 10mg/kg(i.p.) respectively per

day per mouse continued for 10 consecutive days. At the interval of 5 days, the blood parameters were assayed [20].

The remaining 5 groups of mice without tumors were taken to study the effect of complex M4 on hematological parameters. Group 1 were assayed without any treatment and groups 2-4 were treated with complex M4 at an incremental dose of 2.5mg/kg(i.p.), 5.0mg/kg(i.p.)and 10mg/kg(i.p.) respectively per day per mouse. Standard drug bleomycin (0.3mg/kg,i.p.) was injected to group 5.

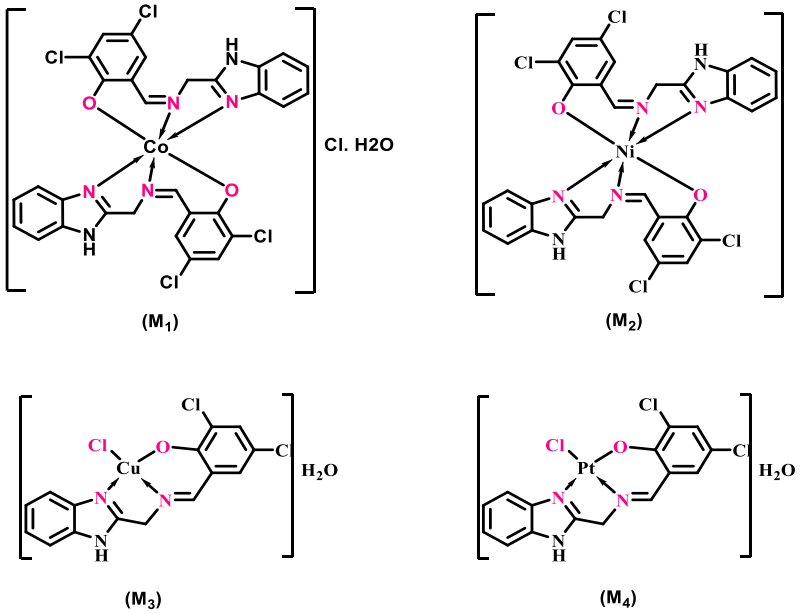


Figure 4. Proposed structure of the complexes M1-M4.

3. Results and Discussion

3.1. Physico-chemical characterization.

All the prepared transition metal complexes are stable at ordinary temperature, and they are insoluble in water, sparingly soluble methanol but completely soluble in coordinated solvents such as DMSO, DMF, and THF. As depicted in Table 1, based on elemental analyses, the synthesized metal transition complexes are formed in the ratio of 2:1 and 1:1 [BMDC : metal]. All the resultant transition complexes were dissolved in DMSO and made with millimolar solution. , The conductance of milli molar solution, was measured at room temperature and compared with the free metal ion solution. The decrease in the molar conductance value in accordance with the formation of complexes and constant conductance at different time intervals reveals that all complexes are stable in the solution state. The presence of chloride ion in the ionic sphere is confirmed by high conductance in M1 complex, while all other complexes are non-electrolyte.

Further, as shown in Table 1, the paramagnetic nature of Schiff base metal complexcontainingCu(II) ion exhibited a magnetic moment between 1.90-1.95 B.M., which is greater than that of the 1.73 (spin-only value). The high spin and low spin complexes of some 3d-series metal ion differ in the quantity of unpaired electron in the complexes when this quantity can be judged more easily from a comparison of the calculated magnetic moment from the low spin and high spin complexes and that of the measured magnetic moment. The number of unpaired electron determination gives information about the oxidation state of the complex containing metal ion. In the meanwhile also establishing geometry of many complexes, the

magnetic moment value (1.92) supported to the square planar geometry around the resultant Cu(II) complex.

Table 1. Elemental analysis, molar conductance and magnetic moment of the ligand and its complexes.

Compounds	C	H	N	O	Conductance of free metal ion in µs	Conductance of respective complexes in µs	Magnetic moment in B.M
BMDC	56.27	03.46	13.12	05.00	-	-	-
M1	48.00	02.95	11.19	06.39	133	96.8	0.0
M2	50.39	03.10	11.75	06.71	146	0.0	3.16
M3	41.11	03.22	09.59	07.30	157	0.0	1.92
M4	32.77	01.83	07.64	02.91	122	0.0	0.0

In the case cobalt complexes, the magnetic moment was not observed, which reveals strong field $d^6 4s^0$ diamagnetic nature of the resultant complex. The spectral data and magnetic moment values were supported to the octahedral geometry for Co(III) complex. The octahedral Ni(II) complexes usually exhibit the magnetic moment value lies in the range 2.91-3.96 B.M [21]. In the resultant Ni(II) complex, the magnetic moment was found to be 3.16 B.M., which reveals that the complex fits in the octahedral geometry. On the other hand, the zero magnetic moments strongly recommends the square planar environment around the Pt(II) ion.

3.2. Electronic absorption spectra.

DMSO solution of the synthesized ligand and its complexes shows (3.0×10^{-6}molL^{-1}), almost similar UV spectra. As depicted in Fig. 5, the bands appeared at 268 nm in the ligand was due to azomethine group and was shifted between 272 and 279 nm for complexes suggesting the involvement of azomethine group in the coordinate bond formation. The complexes exhibited a band at 655 nm due to the transition of $^1A_{1g} \rightarrow {}^1T_{2g}$. All the prepared transition metal complexes attributed the peak at 430 nm due to the possibility of charge transfer transition [22]. The location of the broadband at 457 nm refers to the $^2B_{1g} \rightarrow {}^2E_g$ transitions; it indicates the complexes M1 and M2 belong to the distorted octahedral geometry [23].

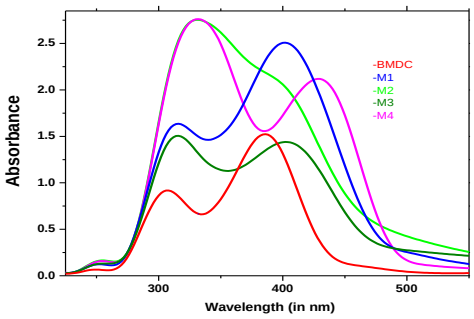


Figure 5. UV-Visible spectra of BMDC and M1–M4 complexes in DMSO solution.

3.3. IR spectral studies.

A band appeared at 1648 cm-^{1} due to the free azomethine group in the heterocyclic ligand, but the band was shifted to a lower value in the complexes indicating its involvement in the coordination [24]. In the IR spectrum of ligand, a band at 1433 cm-^{1} is due to the stretching vibration of C=C group in the benzimidazole ring (Fig. 6). In this section, a representative IR spectrum of complex M2 is depicted and was compared with the synthesized ligand, BMDC. The shifting of these bands in the case of complexes strongly recommends that

the imidazole ring nitrogen is binding with the metal ion, and the bands were located at 1685 cm^{-1} and 1457 cm^{-1}. A medium band at 1267 cm^{-1} due to the vibration of a phenyl hydroxyl group in the Schiff base ligand, but the band is not registered in the prepared complexes, suggesting that the phenolic oxygen atom also acts as a binding site in the resultant complexes. The lower energy region of IR spectra shows bands appearing due to of M-N bond and M-O band at 503 cm^{-1} and 434 cm^{-1}, respectively. Similarly, the IR spectra of other metal complexes under study are depicted in Figs. S1-S3.

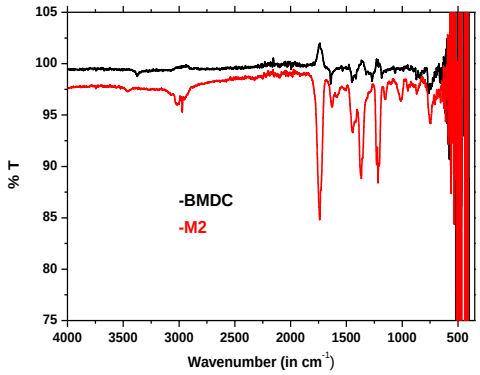


Figure 6. FT-IR spectra of BMDC and M2.

3.4. Thermogravimetric analysis.

All the prepared complexes (M1 - M4) have been subjected to a thermograph over a broad range of temperatures between 27 °C to 800 °C under an inert atmosphere with a heating rate of 10 °C/min.

The thermal investigations of the complexes M1 and M2 are similar. The thermal degradation in M1 complex occurs in two stages (Fig. 7). The initial stage is corresponding to the weight loss of lattice water molecule about 2.88% (calc. 2.517%) at a temperature range of 350-390 °C. Considerable weight loss appears in the second step of degradation due to the departure of two benzimidazole Schiff base molecules with weight loss of 88.54% (calc. 89.55%) between the temperature range 410 and 430 °C. Finally, at the temperature above 445 °C, the stable product left behind as metal oxide.

Similarly, the thermal behavior of the square planar complexes M3 and M4 are similar, while the decomposition appeared in two phases. In the first phase accordance with the loss of lattice water about weight occurs 4.645% (calc. 4.33%) and the temperature range between 215 and 225 °C, followed by major weight loss refers to the decomposition of coordinated chloride as well as a ligand at 300-330 °C, about the mass loss of 85.83% (calc. 85.83%).

3.5. ESR spectra.

The magnetically active copper metal center was investigated by ESR spectral analysis and it was measured on X-band at liquid nitrogen temperature using DMSO as a solvent. ESR spectra of copper complex attributed to the coupling interaction of copper nuclei with the unpaired electron, as a result, causes the appearance of a single main band. The studies of the spectrum give $g_{\parallel} = 2.104$, and $g^{\perp} = 2.043$ in addition to this information clearly understood the trend $g_{\parallel} > g^{\perp} > 2.0023$ in the present case. The analysis indicates that the localization of unpaired electron in Cu(II) ion $dx^2\text{-}y^2$ orbital and is the characteristic spectral feature for axial

symmetry (Fig. 8). Tetragonal elongated geometry strongly recommends for the resultant copper complex [25].

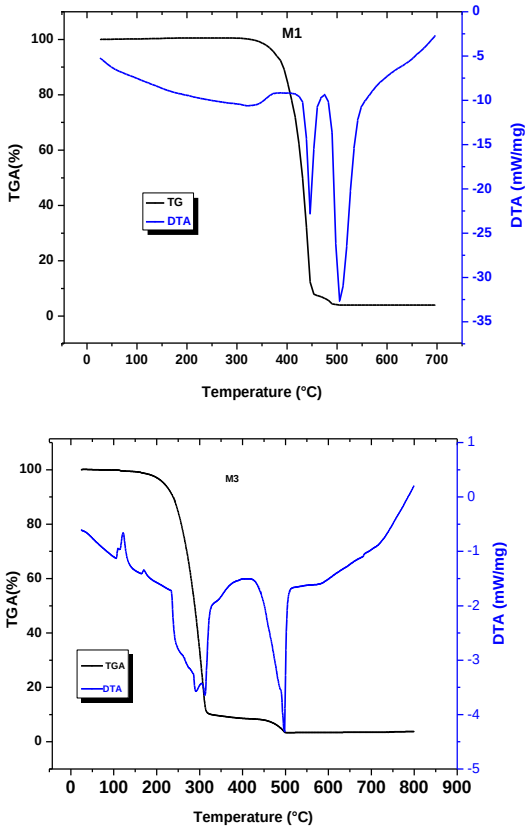


Figure 7. TGA and DTA for M1 (above) and M3 (below).

3.6. Mass spectra.

The formation of M2 complex was conformed via MS technique molecular ion peak was registered at m/z = 696.9686 consistent with its molecular weight; similarly M1 complex exhibited the peak at m/z = 969.9682 due to the formation of coordination sphere it is clearly understood from the mass spectra (Fig. S4-S7) complexes M1 and M2 are in ratio 2:1. The complexes M3 and M4 are in the ratio 1:1 were confirmed from the molecular ion peak of M3 complex attributed at 436.1529, which is complete agreement with theoretical value (436.234) and the complex M4 shows molecular ion peak at m/z = 600.94.

3.7. Antimicrobial activity.

The synthesized ligand (BMDC) and its Co(III), Ni(II), Cu(II) and Pt(II) complexes were screened for their *in vitro* antimicrobial activity by using well diffusion method. The microorganisms were treated with various concentrations of the metal complexes, DMSO as a negative control, and 26 µg of ampicillin was used as a positive control. It is can be seen from Fig. 9 that the considerable range of inhibition zone against Gram-positive bacterial species such as *B. subtilis, S. aureus, L. monocytogenes,* and Gram-negative bacteria's such as *E. coli, V. cholera, P. aeroginosa* at high dilution range from 250 and 100 µg, respectively was observed. All the screened complexes exhibit significant inhibition compared with the parent ligand, BMDC. However, the activity was lower than standard ampicillin. The highest antimicrobial activity was assigned for M4 while M2 was showed the lowest activity, which

confirmed to be active against all the investigated microbial strains. The above result was further supported by the usual mechanism, i.e., Overtone concept and Tweedy's chelation theory [26]. In the case of the chelated complex structure, there is a partial distribution of positively charged metal ion with the ligand atoms of nitrogen and delocalization of electrons found over the entire chelate ring. In this manner, the metal chelating covalent character is enhancing and oblique its permeation via the bacterial membrane layers of lipid and blocking the binding sites of metal in the microorganism.

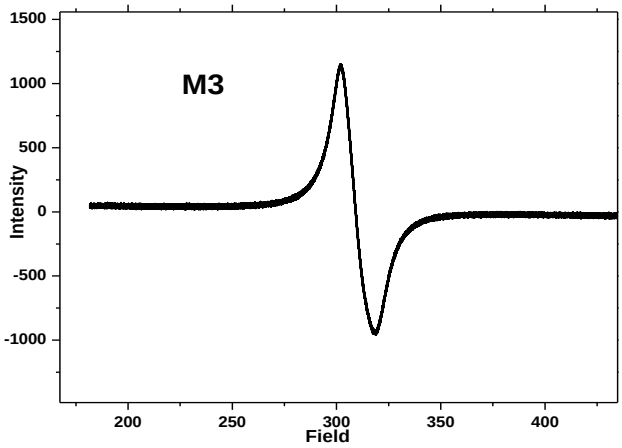


Figure 8. ESR spectrum of M3 complex.

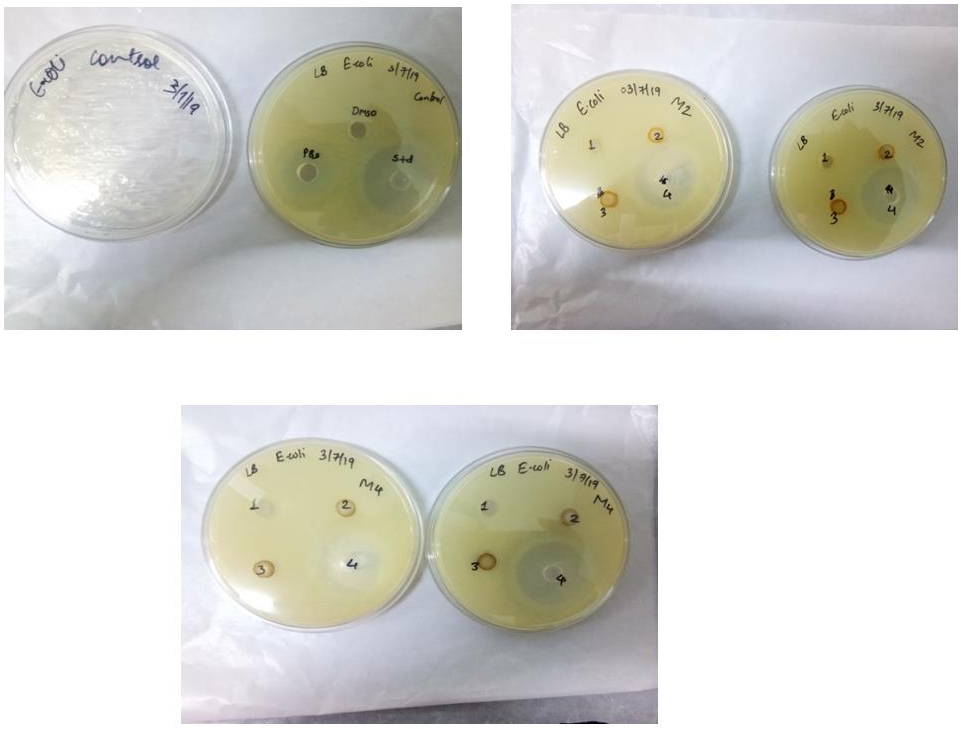


Figure 9. The bactericidal activity of M1 and M4 on *E. coli*.

3.8. Minimum inhibitory concentration.

The minimum inhibitory concentration value was determined for the synthesized ligand BMDC and its complexes. The bacterial culture was allowed to develop overnight, adjusting the concentration, and McFarland turbidity standard was determined.

The serial dilution method was performed to estimate the minimum inhibitory concentration of resultant compounds in the microtitre plate assay. The uncoordinated

benzimidazole imine based derivative exhibited a considerable range of MIC against all bacteria, as shown in Table 2.

Table 2. MIC values of the ligand and its complexes.

Compound	**Concentration range (µg/mL)**					
	Gram-positive bacteria			Gram-negative bacteria		
	B. subtilis	*S. aureus*	*L. monocytogenes*	*E. coli*	*V. cholera*	*P. aeroginosa*
BMDC	>100	>100	>100	>100	>100	>100
M1	65	53	61	53	83	58
M2	91	78	82	69	63	74
M3	52	49	59	48	51	52
M4	38	43	40	37	34	36
Ampicillin	26	26	26	26	26	26

3.9. DNA binding studies.

3.9.1. Electronic absorption titration.

The electronic absorption technique is commonly employed to analyze the binding behavior of metal complexes with DNA. The absorption spectra of prepared metal complexes are investigated in the presence and absence of CT-DNA, as shown (Fig. 10). In the region of UV, two intense bands are observed at 370 and 260 nm, which refers to the ligand to metal charge transfer transition and π-π* transition in the aromatic chromophore. It has been described that the intercalating effect of the complexes constructs on the four important parameters, such as planarity of the ligand, the type of atom donor, the coordination geometry, and the nature of the metal ion or metal atom. Intercalative mode of interaction commonly causes hypochromism and stacking interaction between the DNA base pairs and an aromatic chromophore, which results in the redshift [27]. The degree of redshift and hypochromism are generally constructed with the intercalative binding ability. But, resultant metal complexes that bind non-interactively with CT-DNA may cause hypochromism. In usually, the electronic absorption spectra of metal complexes interact with DNA through intercalation shows considerable red shift and hyphocromism as a result of strong π-π* stacking interaction between the double helix model of DNA base pairs and chromophore of the heterocyclic ligand. In the synthesized complexes, the reduction in absorption intensity (hyphochromism) with the intercalative binding modes of interaction between the metal complexes and the DNA result in a slight redshift.

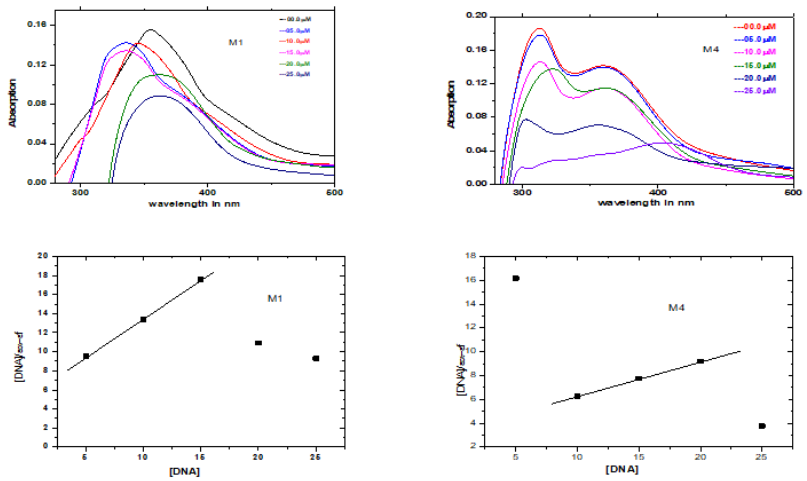


Figure 10. The electronic absorption spectra of metal complexes M1 and M4 in the presence and absence of CT-DNA in tris/NaCl buffer (pH= 7.3). The plot shows [DNA]/ε_a-ε_f versus [DNA].

The result exhibited the successive addition of CT-DNA solution to the solution metal complexes; the significant binding activity was obtained. The values of binding constant K_b of complexes obtained from the intrinsic binding constant equation and the values obtained to be 0.931×10^4, 2.440×10^4, 5.831×10^4 and 8.341×10^4 for transition metal complexes M1, M2, M3, and M4 respectively. The resultant K_b values are lesser than those of typical classical intercalator, ethidium bromide (Kb, 106-107.M-1), and indicate that the binding mechanism of resultant complexes with DNA is always through the intercalative mode.

3.9.2. Fluorescence quenching experiment.

Further, the mode of interaction between CT-DNA and the complexes were confirmed by the EB fluorescence displacement experiment [28]. The fluorescence intensity of EB is very low in the buffer solution due to the fluorescence intensity of Free EB is quenched by solvent molecules, and its emission intensity dramatically enhancing when (placed 10min) it intercalatively combines with CT-DNA. The 12.5 µM of EB concentration and 125 µM of DNA concentration saturates the fluorescence emission intensity. This high intensity is inhibited by the successive treatment of complexes (DNA binding agent) to the system of EB-DNA. In the present study, on increasing the complex concentration, the intensity of DNA-EB complex fluorescence emission quenched gradually (Fig. 11). The intensity of fluorescence emission at 590 nm (excitation at 380 nm) reduced with the concentration of the complexes increase, which is strongly recommended that the complexes could replace DNA bind EB and bound to CT-DNA with the same affinity at the intercalation site [29]. The plot graph Io/I versus [complex] and calculated the Stern-Volmer constant for resultant complexes, The Stern-Volmer constant value was obtained to be 2.141×104, 2.231×104, 2.442×104 & 2.543×104 for M1, M2, M3, and M4 respectively. The higher Ksq value was attributed to the complex M4 due to the presence of square planar platinum complex moiety replacing some EB from the EB-DNA.

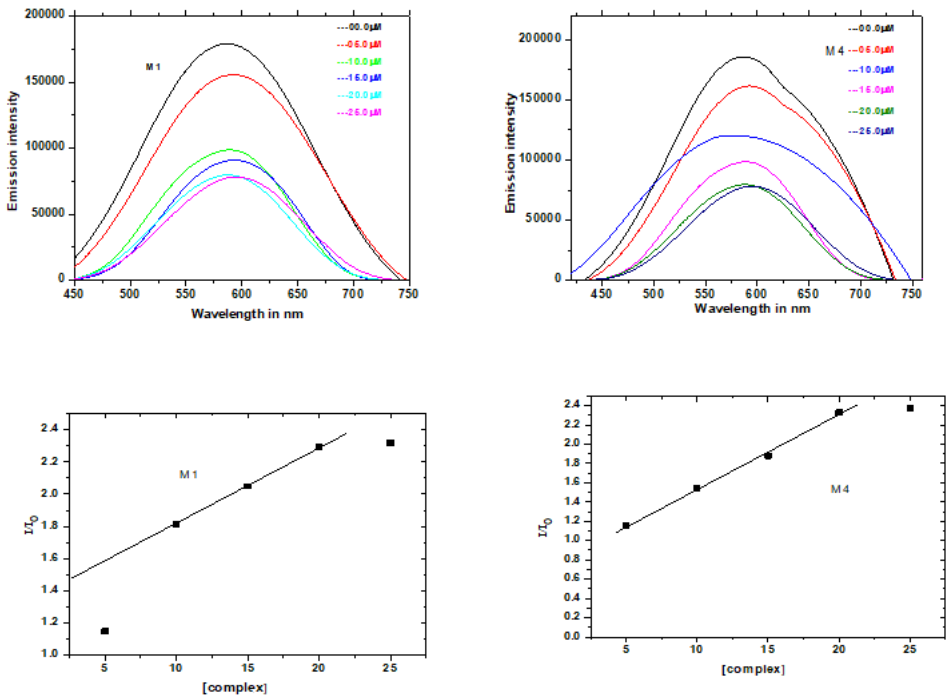


Figure 11. Quenching spectra of EtBr-DNA in the presence of metal complexes M1 and M4 at the concentration 0-25µM in tris/NaCl buffer (pH=7.3). The plot indicates fit of fluorescence intensity versus [complex].

9.2.4. Viscosity measurement.

The viscosity measurements were performed to further analyze the interaction between metal complexes and DNA. Because Optical photophysical methods are not given appropriate clues to support the binding pattern of the metal complexes and DNA. The hydrodynamic measurement that is sensitive to the alter the double-helical conformation of DNA are compliments as least ambiguous and most analytical tests of a binding model in solution in the absence of crystallographic characteristic date. A classical intercalative mode exhibit considerable increase in viscosity of DNA solution due to an increase in isolation of base pairs at intercalation sites, and hence an overall length of the DNA increases [30]. Whereas, the complexes that bind exclusively in the DNA groove by partial or non-classical intercalation under the same condition typically causes a decrease in the viscosity of DNA solution due to kink or bend in the DNA strand and hence, leads to diminishing in its effective length. The value $(\eta/\eta o)^{1/3}$ were plotted against [compound]/[DNA] (Fig. 12). For the resultant complexes, as can be seen, there is an initial decrease in viscosity with an increasing value of [compound]/[DNA] it indicates non-intercalative interaction between DNA and complexes. Later increase of viscosity with increasing the [compound]/[DNA], which indicates a classical intercalation interaction between DNA and synthesized metal complexes.

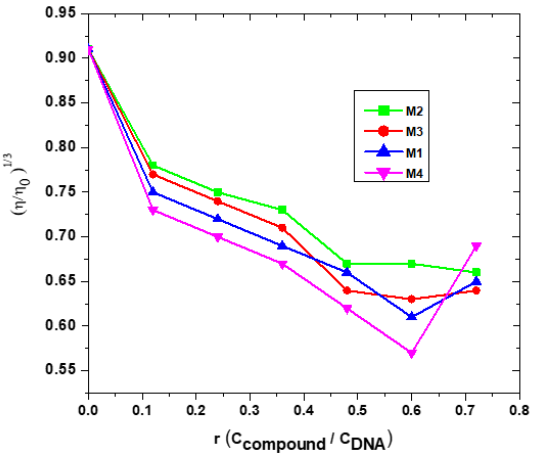


Figure 12. The increasing amount of complexes effect on the relative viscosity of CT-DNA at 28 °C.

9.10. Cleavage activity with pBR322 plasmid DNA.

The gel electrophoresis method was used to study the nuclease efficacy of complex M4. In this method, substrate the supercoiled pBR322 plasmid DNA in the Tris-HCl/50mM NaCl buffer medium at pH=7.3 in the presence of hydrogen peroxide used as mild oxidizing agent and UV-light. When circular plasmid DNA is subjected by electrophoresis, the fastest migration of supercoiled (SC) was occurring in the form I. If spoiled the double-helical conformation of DNA, the supercoiled form will relax to exhibit a slow –migration open circular (OC) form II [31].

The cleavage arrangement of Pt(II) complex as shown in (Fig 13). In the oxidative method control (lane 1), DNA + Complex M4 (lane 2), DNA + hydrogen peroxide (lane 3) do not exhibit any appreciable cleavage of plasmid DNA but shows prominent nuclease activity with successive addition of complex M4 in the presence of hydrogen peroxide (lane 4: DNA + hydrogen peroxide + 20 µM complex M4 and lane 5: DNA + hydrogen peroxide + 40 µM complex M4). The superior cleavage ability showed by complex M4 is due to the intensify the

reaction of platinum ion wit hydrogen peroxide, thereby occurring either diffusible hydroxyl radicals or molecular oxygen, both of which are the ability to alter the double-helical structure of the DNA by Fenton form chemistry [31].

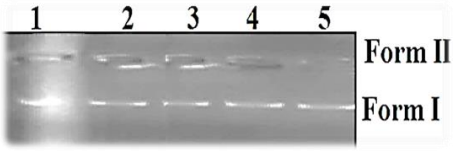


Figure 13. Cleave of pBR 322 DNA by complex M4. Lane 1: DNA + control Plasmid; lane 2: M4 complex alone40µM; lane 3: Hydrogen peroxide (1mM) alone; lane 4: DNA + M4 complex (20µM) + Hydrogen peroxide (1mM); lane 5: DNA + M4 complex (40µM) + Hydrogen peroxide (1 mM).

3.11. Cytotoxic activity of complexes and SAR.

The synthesized heterocyclic ligand and its Co(III), Ni(II), Cu(II) and Pt(II) metal complexes were initially subjected to its *in vitro* cytotoxicity and followed by anti-proliferative activity on varies cell lines such as A549, EAC, SIHA and NIH3T3 together with 5-fluorouracil [32]. The ligand BMDC was alone attributed the cytotoxicity little primarily against EAC with 15µM half inhibitory concentration value (Fig. 14) while after coordination with cobalt, nickel, copper and platinum metal salts, its anti-proliferative efficacy enhanced against A498 cell with a 50% inhibitory concentration of 10µM in MTT cytotoxic assay [32]. The cell viability did not alter by the vehicle control alone. Even though the heterocyclic base itself is a more effective biological active molecule; however, the bonded with aldehyde and metal salts improved its potentiality.

Over the last few decades, chemists are identifying that most of the pharmacologically active drugs contains a heterocyclic system as a part or whole integral entity. Here we are keeping a heterocyclic moiety, which is essential for the clinical activity fused with an aromatic non-heterocyclic system called Benzimidazole Schiff base. These molecules can be exploited into coordination chemistry by utilizing as a ligand for the various metal ions such Co(III), Ni(II), Cu(II), and Pt(II) in order to achieve a more exciting activity in clinical biology. Out of all the four complexes, the Pt(II) complex (M4) displays enhanced clinical properties, which are due to the involvement of platinum metal ion in square planar geometry. Electron distribution is more prominent and less deviation from square planar geometry because all the donor atoms are identical and interact with metal to the same extent.

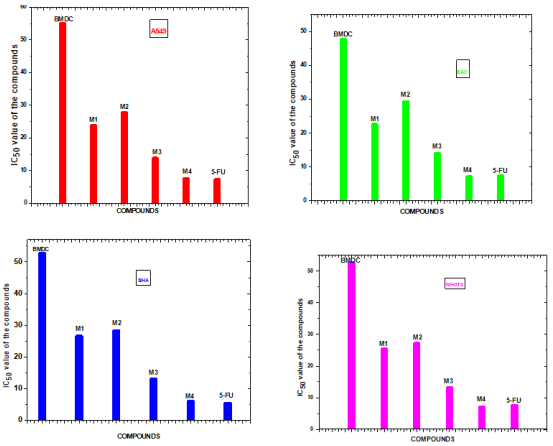


Figure 14. Screening of potent anti-proliferative ligand and complexes in vitro. IC_{50} value of cytotoxic ligand BMDC and complexes (M1, M2, M3 and M4) were determined using MTT assay methods against various cell lines such as A549, EAC, SIHA, and NIH3T3.

3.12. Hematological and serum profile parameters.

Hematological and serum profile parameters of the platinum complex. There are significant changes in hematological parameters after 20th day treatment on EAC cell bearing mice when treated with the complex M4 at a maximum dose of 10mg/kg as compared with EAC control group. Total WBC count was increased in the EAC control cells, whereas alkaline phosphatase urea decreased in EAC bearing groups. These parameters either increase or decrease towards normal value when treated with complex M4 and restored to normal value after 25th day of the treatment (Fig. 15). In comparison with controlled EAC bearing mice, there is a maximum 48.36% reduction of the number of EAC cells when treated with complex M4 at a dose of 10 mg/kg. At a higher dose of complex M4 in normal mice that there is a hike in the number of macrophages and peritoneal cells to some extent.

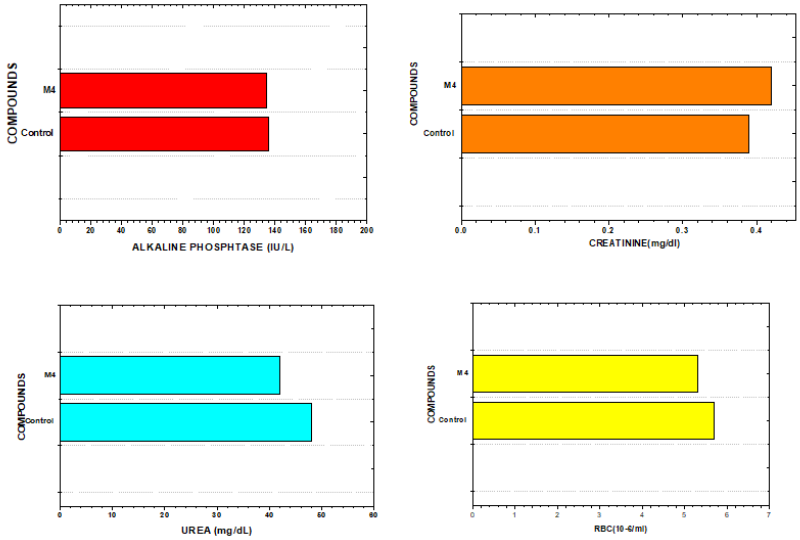


Figure 15. Hematological and serum profiles of the treated M4 normal mice and normal non-tumor bearing mice.

4. Conclusions

The imine based ligand, 2-((E)-((1H-benzo[d]imidazol-2-yl)methylimino)methyl)-4,6-dichlorophenol (BMDC) was synthesized and characterized by a different spectroscopic technique such as NMR, IR, Mass and UV followed by synthesis of Co(III), Ni(II), Cu(II) and Pt(II) complexes. The stoichiometry ratio of metal and ligand of these resultant complexes is 1:2 and 1:1 and characterized them with the help of various spectroscopic techniques as well as conductance measurement. Based on the obained results, an octahedral geometry around the Co(III) and Ni(II) complexes, and square planar geometry around the Cu(II) and Pt(II) complexes have been proposed. All the prepared compounds were screened *in vitro* for antimicrobial activity against various bacterial, and the spectroscopic techniques were used to conform the binding activity towards the CT-DNA. The spectral studies reveal that the CT-DNA interacts with resultant metal complexes in an intercalactive mode. The cleavage activity with pBR322 DNA was done by the gel electrophoretic method.

Funding

This research received no external funding.

Acknowledgments

The author sincerely thankful to IIT Bombay and IOE, University of Mysore for their kind co-operation in the characterization of metal complexes. SPK thankfully acknowledges the Director, Amrita Vishwa Vidyapeetham, Mysuru Campus, Mysuru, for infrastructural facilities.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Vasava, M.S.; Bhoi, M.N.; Rathwa, S.K.; Jethava, D.J.; Acharya, P.T.; Patel, D.B.; Patel, H.D. Benzimidazole: A Milestone in the Field of Medicinal Chemistry. *Mini Reviews in Medicinal Chemistry* **2020**, *20*, 532-565, https://doi.org/10.2174/1389557519666191122125453.
2. Bansal, Y.; Kaur, M.; Bansal, G. Antimicrobial Potential of Benzimidazole Derived Molecules. *Mini Rev Med Chem* **2019**, *19*, 624-646, https://doi.org/10.2174/138955751.
3. Ndagi, U.; Mhlongo, N.; Soliman, M.E. Metal complexes in cancer therapy - an update from drug design perspective. *Drug design, development and therapy* **2017**, *11*, 599-616, https://doi.org/10.2147/DDDT.S119488.
4. Dasari, S.; Bernard Tchounwou, P. Cisplatin in cancer therapy: Molecular mechanisms of action. *European Journal of Pharmacology* **2014**, *740*, 364-378, https://doi.org/10.1016/j.ejphar.2014.07.025.
5. Hazra, M.; Dolai, T.; Pandey, A.; Dey, S.K.; Patra, A. Synthesis and Characterisation of Copper(II) Complexes with Tridentate NNO Functionalized Ligand: Density Function Theory Study, DNA Binding Mechanism, Optical Properties, and Biological Application. *Bioinorganic Chemistry and Applications* **2014**, *2014*, 1-13, https://doi.org/10.1155/2014/104046.
6. Savithri, K.; Kumar, B.C.V.; Vivek, H.K.; Revanasiddappa, H.D. Synthesis and Characterization of Cobalt(III) and Copper(II) Complexes of 2-((E)-(6-Fluorobenzo[d]thiazol-2-ylimino) methyl)-4-chlorophenol: DNA Binding and Nuclease Studies—SOD and Antimicrobial Activities. *International Journal of Spectroscopy* **2018**, *2018*, 1-15, https://doi.org/10.1155/2018/8759372.
7. Thamilarasan, V.; Sengottuvelan, N.; Sudha, A.; Srinivasan, P.; Chakkaravarthi, G. Cobalt(III) complexes as potential anti-cancer agents: Physicochemical, structural, cytotoxic activity and DNA/protein interactions. *Journal of Photochemistry and Photobiology B: Biology* **2016**, *162*, 558-569, https://doi.org/10.1016/j.jphotobiol.2016.06.024.
8. Skyrianou, K.C.; Psycharis, V.; Raptopoulou, C.P.; Kessissoglou, D.P.; Psomas, G. Nickel–quinolones interaction. Part 4 — Structure and biological evaluation of nickel(II)–enrofloxacin complexes compared to zinc(II) analogues. *Journal of Inorganic Biochemistry* **2011**, *105*, 63-74, https://doi.org/10.1016/j.jinorgbio.2010.09.007.
9. Jin, Y.; Lewis, M.A.; Gokhale, N.H.; Long, E.C.; Cowan, J.A. Influence of Stereochemistry and Redox Potentials on the Single- and Double-Strand DNA Cleavage Efficiency of Cu(II)· and Ni(II)·Lys-Gly-His-Derived ATCUN Metallopeptides. *Journal of the American Chemical Society* **2007**, *129*, 8353-8361, https://doi.org/10.1021/ja0705083.
10. Shahabadi, N.; Mohammadi, S. Synthesis Characterization and DNA Interaction Studies of a New Zn(II) Complex Containing Different Dinitrogen Aromatic Ligands. *Bioinorganic Chemistry and Applications* **2012**, *2012*, 1-8, https://doi.org/10.1155/2012/571913.
11. Basappa Chidananda, V.K.; Ramakrishna, D.; Kaur, M.; Hosakere Doddarevanna, R. Benzimidazolyl based Schiff base palladium complex in an ionic liquid: an effective combination for Suzuki coupling. *Journal of Coordination Chemistry* **2017**, *70*, 1573-1584, https://doi.org/10.1080/00958972.2017.1311412.
12. Ramu, R.; Shirahatti, P.; Zameer, F.; Bhadrapura Lakkappa, D.; Prasad M N, N. Evaluation of banana (Musa sp. var. Nanjangud rasa bale) flower and pseudostem extracts on antimicrobial, cytotoxicity and thrombolytic activities. *International Journal of Pharmacy and Pharmaceutical Sciences* **2015,** *7*, 136-140.
13. Suntharalingam, K.; Mendoza, O.; Duarte, A.A.; Mann, D.J.; Vilar, R. A platinum complex that binds non-covalently to DNA and induces cell death via a different mechanism than cisplatin. *Metallomics* **2013**, *5*, 514-523, https://doi.org/10.1039/c3mt20252f.
14. Kumaravel, G.; Ponya Utthra, P.; Raman, N. Exploiting the biological efficacy of benzimidazole based Schiff base complexes with l-Histidine as a co-ligand: Combined molecular docking, DNA interaction, antimicrobial and cytotoxic studies. *Bioorganic Chemistry* **2018**, *77*, 269-279, https://doi.org/10.1016/j.bioorg.2018.01.024.
15. Alizadeh, R.; Yousuf, I.; Afzal, M.; Srivastav, S.; Srikrishna, S.; Arjmand, F. Enantiomeric fluoro-substituted benzothiazole Schiff base-valine Cu(II)/Zn(II) complexes as chemotherapeutic agents: DNA

binding profile, cleavage activity, MTT assay and cell imaging studies. *Journal of Photochemistry and Photobiology B: Biology* **2015**, *143*, 61-73, https://doi.org/10.1016/j.jphotobiol.2014.12.027.
16. Schildkraut, C.L.; Marmur, J.; Doty, P. The formation of hybrid DNA molecules and their use in studies of DNA homologies. *Journal of Molecular Biology* **1961**, *3*, 595-IN516, https://doi.org/10.1016/S0022-2836(61)80024-7.
17. Mallesh, H.B.; Krishnamurthy, G.; Shashikala, N. Reactions of 1-p-dimethylaminobenzyl-2-p-dimethylaminophenylbenzimidazole with cobalt(II), zinc(II) and cadmium(II) salts. *Asian Journal of Chemistry* **2004**, *16*, 1439-1446.
18. Prabhakar, B.T.; Khanum, S.A.; Jayashree, K.; Salimath, B.P.; Shashikanth, S. Anti-tumor and proapoptotic effect of novel synthetic benzophenone analogues in Ehrlich ascites tumor cells. *Bioorganic & Medicinal Chemistry* **2006**, *14*, 435-446, https://doi.org/10.1016/j.bmc.2005.08.039.
19. Malojirao, V.H.; Vigneshwaran, V.; Thirusangu, P.; Mahmood, R.; Prabhakar, B.T. The tumor antagonistic steroidal alkaloid Solanidine prompts the intrinsic suicidal signal mediated DFF-40 nuclear import and nucleosomal disruption. *Life Sciences* **2018**, *199*, 139-150, https://doi.org/10.1016/j.lfs.2018.03.015.
20. Ali, S.M.M.; Zakir, H.M.; Shahriar, S.; Sarkar, M.; Dey, A.K.; Nur, H.P.; Jesmin, M. In vivo anti-cancer activities of Ni (II)-Benzoin thiosemicarbazone complex [Ni(BTSC)2] against ehrlich ascites carcinoma cells. *Journal of Bio-Science* **2018**, *23*, 77-88.
21. Jha, N.N.; Ray, I.P. Magnetic studies on Co(II) and Ni(II) complexes of hydroxamic acid. *Asian Journal of Chemistry* **2000**, *12*, 703-706.
22. Shakya, R.; Imbert, C.; Hratchian, H.P.; Lanznaster, M.; Heeg, M.J.; McGarvey, B.R.; Allard, M.; Schlegel, H.B.; Verani, C.N. Structural, spectroscopic, and electrochemical behavior of trans-phenolato cobalt(iii) complexes of asymmetric NN′O ligands as archetypes for metallomesogens. *Dalton Transactions* **2006**, 21, 2517-2525, https://doi.org/10.1039/B514190G.
23. Raman, N.; Ravichandran, S.; Thangaraja, C. Copper(II), cobalt(II), nickel(II) and zinc(II) complexes of Schiff base derived from benzil-2,4-dinitrophenylhydrazone with aniline. *Journal of Chemical Sciences* **2004**, *116*, 215-219, https://doi.org/10.1007/BF02708270.
24. Ommenya, F.K.; Nyawade, E.A.; Andala, D.M.; Kinyua, J. Synthesis, Characterization and Antibacterial Activity of Schiff Base, 4-Chloro-2-{(E)-[(4-Fluorophenyl)imino]methyl}phenol Metal (II) Complexes. *Journal of Chemistry* **2020**, *2020*, https://doi.org/10.1155/2020/1745236.
25. Kivelson, D.; Neiman, R. ESR Studies on the Bonding in Copper Complexes. *The journal of chemical physics* **1961,** *149*, 1-8, https://doi.org/10.1063/1.1731880.
26. Sultan, J.S.; Lateaf, S.M.; Rashid, D.K. Synthesis, Characterization and Antibacterial Activity of Mixed Ligand (HL) Complexes Mn(ll), Co(ll), Ni(ll), Zn(ll), Cd(ll) and Hg(ll) with Azide (N3 −). *Open Journal of Inorganic Chemistry* **2015,** *5*, 102-111, http://dx.doi.org/10.4236/ojic.2015.54011.
27. Loganathan, R.; Ramakrishnan, S.; Ganeshpandian, M.; Bhuvanesh, N.S.P.; Palaniandavar, M.; Riyasdeen, A.; Akbarsha, M.A. Mixed ligand copper(ii) dicarboxylate complexes: the role of co-ligand hydrophobicity in DNA binding, double-strand DNA cleavage, protein binding and cytotoxicity. *Dalton Transactions* **2015**, *44*, 10210-10227, https://doi.org/10.1039/C4DT03879G.
28. Raja, A.; Rajendiran, V.; Uma Maheswari, P.; Balamurugan, R.; Kilner, C.A.; Halcrow, M.A.; Palaniandavar, M. Copper(II) complexes of tridentate pyridylmethylethylenediamines: Role of ligand steric hindrance on DNA binding and cleavage. *Journal of Inorganic Biochemistry* **2005**, *99*, 1717-1732, https://doi.org/10.1016/j.jinorgbio.2005.05.014.
29. Baguley, B.C.; Le Bret, M. Quenching of DNA-ethidium fluorescence by amsacrine and other antitumor agents: a possible electron-transfer effect. *Biochemistry* **1984**, *23*, 937-943, https://doi.org/10.1021/bi00300a022.
30. Chauhan, M.; Banerjee, K.; Arjmand, F. DNA Binding Studies of Novel Copper(II) Complexes Containing l-Tryptophan as Chiral Auxiliary: In Vitro Antitumor Activity of Cu−Sn2 Complex in Human Neuroblastoma Cells. *Inorganic Chemistry* **2007**, *46*, 3072-3082, https://doi.org/10.1021/ic061753a.
31. Uma, V.; Kanthimathi, M.; Weyhermuller, T.; Nair, B.U. Oxidative DNA cleavage mediated by a new copper (II) terpyridine complex: Crystal structure and DNA binding studies. *Journal of Inorganic Biochemistry* **2005**, *99*, 2299-2307, https://doi.org/10.1016/j.jinorgbio.2005.08.011.
32. Gurupadaswamy, H.D.; Girish, V.; Kavitha, C.V.; Raghavan, S.C.; Khanum, S.A. Synthesis and evaluation of 2,5-di(4-aryloylaryloxymethyl)-1,3,4-oxadiazoles as anti-cancer agents. *European Journal of Medicinal Chemistry* **2013**, *63*, 536-543, https://doi.org/10.1016/j.ejmech.2013.02.040.