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Synthesis, Characterization and DNA cleavage studies of Isomeric Pyridyl-Tetrazole Ligands and their Ni(II) and Zn(II) Complexes

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Abstract

A new series of Ni(II) and Zn(II) complexes were synthesized from bidentate isomeric pyridyl tetrazole ligands such as 2-(1-vinyl-1H-tetrazol-5-yl)pyridine (L\textsuperscript{1}), N,N-dimethyl-3-(5-(pyridin-2-yl)-1H-tetrazol-1-yl)propan-1-amine(L\textsuperscript{2}), 2-(2-vinyl-2H-tetrazol-5-yl)pyridine(L\textsuperscript{3}), N,N-dimethyl-3-(5-(pyridin-2-yl)-2H-tetrazol-2-yl)propan-1-amine (L\textsuperscript{4}). All the complexes were characterized by the elemental analysis, molar conductance, FTIR, UV-VIS and magnetic studies. The conductance and spectroscopic data suggested that, the ligands act as monobasic bidentate ligands and form octahedral complexes with general formula [M(L\textsuperscript{1−4})\textsubscript{2}Cl\textsubscript{2}], (M= Ni(II) and Zn(II)). In addition metal complexes displayed good antioxidant and moderate nematicidal activities. The cytotoxicity of ligands and their metal complexes have been evaluated by MTT assay. The DNA cleavage activity of the metal complexes was performed using agarose gel electrophoresis in the presence and absence of oxidant H\textsubscript{2}O\textsubscript{2}. All metal complexes showed significant nuclease activity in the presence of H\textsubscript{2}O\textsubscript{2}.

Keywords: Isomeric Pyridyl-tetrazole derivative ligands; Nickel and Zinc complexes; DNA Cleavage studies.
Introduction

Biologically active Isomeric pyridyl tetrazole derivatives have been under great investigations as part of inorganic chemistry. Polyazole rings are versatile ligands[1] for coordinating transition metals, therefore synthesis of transition metal complexes containing polyazole rings, particularly tetrazoles and their derivatives have given enormous significance, due to their practical applications [2–4]. There is an increasing interest of tetrazole derivatives for the development of ‘‘click’’ chemistry which was reported by Sharpless and co-workers [5]. Conversely, tetrazole-based compounds have made known special functionalities with interesting structures [6]. Tetrazole derivatives have found applications in therapeutics as antihypertensive agents [7], antibiotics [8] and drugs for AIDS treatment [9].

Even though many tetrazole containing derivatives are available in the literature, there is always an increasing demand for the development of novel and effective tetrazole containing therapeutic agents. In continuation of our ongoing research on DNA binding and cleavage activities of transition metal complexes [10], in this paper we presented the synthesis, characterization and DNA cleavage activities of Ni(II) and Zn(II) complexes which are obtained by the reaction of pyridyl-tetrazole derivatives which contain pendant arms like vinyl or propyl-N(CH\textsubscript{3})\textsubscript{2} group.

Experimental Materials and measurement

Chemicals were purchased from Sigma-Aldrich and metals used in the preparation of the complexes are of reagent grade. The solvents used in the synthesis of the ligands and metal complexes were distilled before use. All other chemicals were of AR grade and were used without further purification. The elemental analysis of carbon, hydrogen, and nitrogen contents was performed using Perkin Elmer CHNS analyser. Molar conductance of the complexes was measured using a Digisun conductivity meter in DMF. The electronic
absorption spectra of the complexes were recorded on JASCO V-670 Spectrophotometer in the wavelength region of 250–1400 nm in the solid state. The FTIR spectra of the complexes were recorded on Tensor 2 FTIR spectrophotometer in the region of 4000–400 cm\(^{-1}\) using KBr disc. The magnetic susceptibilities of Ni(II) complexes were measured with a Sherwood scientific balance. Diamagnetic corrections were calculated from Pascal’s constants. The magnetic moment values were calculated using the relation \(\mu_{\text{eff}} = 2.83 (\chi m T)^{1/2}\) B.M.

2.3. Synthesis of ligands.

2.3.1. ligands

The preparation of \(L\) is carried as per literature [11] M.p. 221–223°C. C, 48.98; H, 3.43; N, 47.60; \(^1\)H NMR (CD\(_3\)OD): 8.56 (d, 1H, J = 7.9 Hz, pyr-H), 8.0 (d, 1H, J = 7.8 Hz, pyr-H), 7.79 (t, 1H, J = 7.8 Hz, pyr-H), 7.26 (t, 1H, J = 7.9 Hz, pyr-H), 7.1 (s, 1H, tetrazole-H) ppm.

![Scheme-1](image.png)

**Scheme-1.** Synthetic route for ligands \(L^1-L^4\), reaction conditions (i) \(\text{NaN}_3, \text{LiCl}, \text{NH}_4\text{Cl, DMF, reflux 10 h;}\) (ii) \(\text{K}_2\text{CO}_3, \text{acetonitrile, reflux 24 h.}\)

2.3.1.a. \(N,N\)-dimethyl-3-(5-(pyridin-2-yl)-1H-tetrazol-1-yl)propan-1-amine(\(L^2-L^4\)):
To the compound L (1.0 gm, 6.8 mmol) dissolved in acetonitrile (30 mL) was added potassium carbonate (4.6 gm, 34 mmol). The resulting solution was refluxed for 30 min and to hot solution was added 3-chloro-N,N-dimethylpropan-1-amine (3.1 gm, 22 mmol). The reaction mixture was then stirred at reflux temperature for a further 24 h. After cooling, the solvent was removed under reduced pressure to afford a white precipitate, which was purified by column chromatography on silica gel (Ethyl acetate: Hexane by the ratio of 20:80) to give isomers L\textsuperscript{1} and L\textsuperscript{3}.

### 2.3.1. a. Synthesis of 2-(1-vinyl-1H-tetrazol-5-yl)pyridine:

The above procedure is followed with 2-chlorovinyl (2.0 gm, 24 mmol) to give L\textsuperscript{1} and L\textsuperscript{3}.

\textbf{L\textsuperscript{1}}: brown White needles, (0.35 g, yield 27%). M.p. 57–59 °C. Anal. Calc. for C\textsubscript{8}H\textsubscript{7}N\textsubscript{5} (173.17): C, 55.48; H, 4.07; N, 40.44; \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 300 MHz): \(\delta = 8.70\) (d, 1H, J = 4.8 Hz, pyr-H), 8.37 (d, 1H, J = 7.8 Hz, pyr-H), 7.86 (dt, 1H, J = 7.8, 1.5 Hz, pyr-H), 7.53 (dd, 1H, J = 6.9, 5.1 Hz, pyr-H), 5.32(t, 1H, J=6.9,vinyl-H), 4.21-4.18 (d, 2H, J=6.9, Vinyl-2H).

\textsuperscript{13}C NMR (CDCl\textsubscript{3}, 60 MHz): \(\delta 152.15\) (CN\textsubscript{4}), 149.73, 144.39, 138.14, 125.79, 124.35, 127.3 (HC,vinyl), 102.9 ppm (2H, vinyl).

\textbf{L\textsuperscript{3}}: brown White needles, (0.35 g, yield 27%). M.p. 57–58 °C. Anal. Calc. for C\textsubscript{8}H\textsubscript{7}N\textsubscript{5} (173.17): C, 55.48; H, 4.07; N, 40.44; \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 300 MHz): \(\delta = 8.70\) (d, 1H, J = 4.8 Hz, pyr-H), 8.37 (d, 1H, J = 7.8 Hz, pyr-H), 7.86 (dt, 1H, J = 7.8, 1.5 Hz, pyr-H), 7.53 (dd, 1H, J = 6.9, 5.1 Hz, pyr-H), 5.31(t, 1H, J=6.9,vinyl-H), 4.21-4.18 (d, 2H, J=6.9, Vinyl-2H).

\textsuperscript{13}C NMR (CDCl\textsubscript{3}, 60 MHz): \(\delta 151.15\) (CN\textsubscript{4}), 149.73, 144.39, 138.14, 125.79, 124.35, 127.3 (HC,vinyl), 102.9 ppm (2H, vinyl).

\textbf{L\textsuperscript{2}}: white brown solid (0.55 g, yield 26%). M.p. 72–76 °C. Anal. Calc. for C\textsubscript{11}H\textsubscript{16}N\textsubscript{6} (232.28): C, 56.88; H, 6.94; N, 36.18; \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 300 MHz) : \(\delta 8.71\) (d, 1H, J = 4.5 Hz, pyr-H), 8.37 (d, 1H, J = 8.1 Hz, pyr-H), 7.91 (dt, 1H, J = 7.8, 1.8 Hz, pyr-H), 7.45 (dd, 1H, J = 7.5, 5.1 Hz, pyr-H), 5.12 (t, 2H, J = 6.9 Hz, CH\textsubscript{2}N), 2.88 (t, 2H, J = 6.9 Hz, CH\textsubscript{2}), 2.42(p, 2H, J =
6.9 Hz, CH₂), 2.29 (s, 6H, N(CH₃)₂) ppm. ¹³C-NMR (CDCl₃, 60 MHz): δ 150.45 (CN₄), 148.53, 144.66, 137.16, 123.15, 122.24, 48.24 (CH₂N₄), 43.56 (CH₂N-(CH₃)₂), 26.82, ppm.

L⁴: white brown solid (0.55 g, yield 26%). M.p. 66-68°C. Anal. Calc. for C₁₁H₁₆N₆ (232.28): C, 56.88; H, 6.94; N, 36.18; ¹H NMR (CDCl₃, 300 MHz): δ 8.65 (d, 1H, J = 4.5 Hz, pyr-H), 8.28 (d, 1H, J = 8.1 Hz, pyr-H), 7.87 (dt, 1H, J = 7.8, 1.8 Hz, pyr-H), 7.34 (t, 1H, J = 7.5, 5.1 Hz, pyr-H), 5.06 (t, 2H, J = 6.9 Hz, CH₂N), 2.82 (t, 2H, J = 6.9 Hz, CH₂), 2.42(p, 2H, J = 6.9 Hz, CH₂), 2.27 (s, 6H, N(CH₃)₂) ppm. ¹³C-NMR (CDCl₃, 60 MHz): δ 151.35 (CN₄), 149.44, 145.36, 138.08, 124.64, 122.12, 49.54 (CH₂N₄), 44.34 (CH₂N-(CH₃)₂), 26.82, ppm.

**Synthesis of complexes**

The appropriate ligand (L¹-L⁴) (1.36 mol) was dissolved in methanol (30 mol) and added to a MCl₂·H₂O (1.36 mol) methanol solution (10 mol). The resulting pale green to green coloured solutions were then heated to reflux for 2-3 h; the solution was left over night at room temperature and filtered to collect respective precipitate.
Scheme 2. Synthesis of 1N and 2N –substituted Nickel and Zinc, pyridyl-tetrazole ligands tetrazole complexes (1- 4)

[Ni(L^1)_2]Cl_2: Dark Green solid (0.12 g, yield 28%) Anal. Calc. for C_{16}H_{14}Cl_2N_{10}Ni (475.95): C, 40.38; H, 2.96; N, 29.43; Found: C, 40.76; H, 2.98; N, 29.96% IR (KBr): ν = 3245, 2945, 1648, 1594, 1496, 1196, 1147, 1023, 835, 785 cm\(^{-1}\). \(\lambda_{\max}\) (MeOH) 376 nm, \(\epsilon = 40\) M\(^{-1}\)cm\(^{-1}\).
Magnetic moment: 3.2 B.M.
[Ni(L²)₂Cl₂]: Pale Green crystals (0.14 g, yield 32%) Anal. Calc. for C₁₆H₁₄Cl₂N₁₀Ni (475.95): C, 40.38; H, 2.96; N, 29.43; Found: C, 40.76; H, 2.98; N, 29.96% IR (KBr): v = 3245, 2945, 1648, 1594, 1496, 1196, 1147, 1023, 835, 785 cm⁻¹. λ_max (MeOH) 388 nm, ε = 38 M⁻¹cm⁻¹. Magnetic moment: 3.2 B.M.

[Zn(L¹)₂Cl₂]: Waxy cream solid (0.17 g, 56%). C₁₆H₁₄Cl₂N₁₀Zn(482.64): Calc. C, 39.82; H, 2.92; N, 29.02; Found: C, 26.92; H, 2.63; N, 17.06%. IR (KBr): m = 2945, 2823, 1610, 1565, 1548, 1453, 1058, 1015, 850, 770 cm⁻¹. 1H NMR (CDCl₃): δ = 8.78 (m, 1H, pyr-H), 8.35 (m, 1H, pyr-H), 7.95 (m, 1H, pyr-H), 7.45 (m, 1H, pyr-H), 3.49 (d, 2H, J = 6.2 Hz, vinyl CH₂), 2.18 (t, 1H, J = 6.2 Hz vinyl CH) ppm. ¹³C NMR (CDCl₃): δ 152.15 (CN₄), 149.73, 144.39, 138.14, 125.79, 124.35, 127.3 (HC, vinyl), 102.9 ppm (2H, vinyl).

[Zn(L³)₂Cl₂]: Waxy orange solid (0.19 g, 58%). C₁₆H₁₄Cl₂N₁₀Zn(482.64): Calc. C, 39.82; H, 2.92; N, 29.02; Found: C, 26.92; H, 2.63; N, 17.06%. IR (KBr): m = 2965, 2833, 1612, 1568, 1548, 1453, 1058, 1015, 850, 780 cm⁻¹. 1H NMR (CDCl₃): δ = 8.68 (m, 1H, pyr-H), 8.45 (m, 1H, pyr-H), 7.95 (m, 1H, pyr-H), 7.45 (m, 1H, pyr-H), 3.49 (d, 2H, J = 6.2 Hz, vinyl CH₂), 2.18 (t, 1H, J = 6.2 Hz vinyl CH) ppm. ¹³C NMR (CDCl₃): δ 153.15 (CN₄), 149.73, 145.39, 138.14, 125.79, 124.35, 127.3 (HC, vinyl), 102.9 ppm (2H, vinyl).

[Ni(L²)₂Cl₂]: Pale Green precipitate (0.12 g, yield 32%) Anal. Calc. for C₂₂H₃₂Cl₂N₁₂Ni (594.17): C, 44.47; H, 5.43; N, 28.29; Found: C, 44.36; H, 5.33; N, 28.96% IR (KBr): v = 3245, 2945, 1648, 1594, 1496, 1196, 1147, 1023, 835, 785 cm⁻¹. λ_max (MeOH) 346 nm, ε = 86 M⁻¹cm⁻¹. Magnetic moment: 3.2 B.M.

[Ni(L³)₂Cl₂]: Pale Green precipitate (0.13 g, yield 42%) Anal. Calc. for C₂₂H₃₂Cl₂N₁₂Ni (594.17): C, 44.47; H, 5.43; N, 28.29; Found: C, 44.34; H, 5.32; N, 28.86% IR (KBr): v = 3235, 2925, 1648, 1594, 1496, 1196, 1157, 1023, 835, 785 cm⁻¹. λ_max (MeOH) 366 nm, ε = 52 M⁻¹cm⁻¹. Magnetic moment: 3.2 B.M.
[Zn(L^2)_2]Cl_2: Waxy cream solid (0.17 g, 56%). C_{22}H_{32}Cl_2N_{12}Zn (600.86): Calc. C, 43.98; H, 5.37; N, 27.97; Found: C, 43.92; H, 5.33; N, 28.36%. IR (KBr): m = 2955, 2823, 1610, 1545, 1548, 1453, 1058, 1015, 850, 770 cm\(^{-1}\). \(^1\)H NMR (CDCl₃, 300 MHz) : \(\delta\) 8.71 (d, 1H, \(J = 4.5\) Hz, pyr-H), 8.37 (d, 1H, \(J = 8.1\) Hz, pyr-H), 7.91 (dt, 1H, \(J = 7.8, 1.8\) Hz, pyr-H), 7.45 (dd, 1H, \(J = 7.5, 5.1\) Hz, pyr-H), 5.12 (t, 2H, \(J = 6.9\) Hz, CH₂N), 2.88 (t, 2H, \(J = 6.9\) Hz, CH₂), 2.42(p, 2H, \(J = 6.9\) Hz, CH₂), 2.29 (s, 6H, N(CH₃)₂) ppm. \(^{13}\)C-NMR (CDCl₃, 60 MHz): \(\delta\) 150.45 (CN₄), 148.53, 144.66, 137.16, 123.15, 122.24, 48.24 (CH₂N₄), 43.56 (CH₂N-(CH₃)₂), 26.82, ppm.

[Zn(L^4)_2]Cl_2: cream solid (0.18 g, 46%). C_{22}H_{32}Cl_2N_{12}Zn (600.86): Calc. C, 43.98; H, 5.37; N, 27.97; Found: C, 43.92; H, 5.33; N, 28.36%. IR (KBr): m = 2965, 2833, 1610, 1545, 1548, 1453, 1058, 1015, 850, 770 cm\(^{-1}\). \(^1\)H NMR (CDCl₃, 300 MHz) : \(\delta\) 8.61 (d, 1H, \(J = 4.5\) Hz, pyr-H), 8.37 (d, 1H, \(J = 8.1\) Hz, pyr-H), 7.91 (dt, 1H, \(J = 7.8, 1.8\) Hz, pyr-H), 7.45 (dd, 1H, \(J = 7.5, 5.1\) Hz, pyr-H), 5.12 (t, 2H, \(J = 6.9\) Hz, CH₂N), 2.88 (t, 2H, \(J = 6.9\) Hz, CH₂), 2.42(p, 2H, \(J = 6.9\) Hz, CH₂), 2.29 (s, 6H, N(CH₃)₂) ppm. \(^{13}\)C-NMR (CDCl₃, 60 MHz): \(\delta\) 151.45 (CN₄), 148.53, 144.66, 137.16, 123.15, 122.24, 48.24 (CH₂N₄), 43.56 (CH₂N-(CH₃)₂), 26.82, ppm.

2.5. Nematicidal Activity.

Root knot nematode, *Meloidogyne incognita*, is major plant parasitic nematodes affecting quantity and quality of the crop production in many annual and perennial crops. *Meloidogyne* nematode can develop galls and lesions in the roots, thereby causing stunted growth of the plants. Some of the chemicals can be used to control nematodes [12]. Nematicidal activity of the complexes was carried out on *Meloidogyne incognita*. Fresh egg masses of *Meloidogyne incognita* are collected from stock culture maintained on tomato (*Lycopersicon esculentum*) root tissues and kept in water for egg hatching. The eggs suspensions were poured on a cotton wool filter paper and incubated at 30°C to obtain freshly hatched juveniles (J2). Juveniles
collected within 48 h were used for screening nematicidal activity of the compounds. The compounds were initially dissolved in dimethyl sulfoxide (DMSO) and then in distilled water to make dilutions of 250, 150, and 50 µg/mL. Experiments were performed under laboratory conditions at 30°C. About 100 freshly hatched second stage juveniles were suspended in 5mL of each diluted compound and incubated. Distilled water with nematode larvae was taken as control. The dead nematodes were observed under an inverted binocular microscope. After an incubation of 24 and 48 h, percentage of mortality was calculated. Nematodes were considered dead if they did not move when probed with a fine needle [13].

2.6. DPPH Radical Scavenging Activity.

The free radical scavenging activities of the metal complexes were determined by using DPPH free radical scavenging method according to the literature [14]. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. In the spectrophotometric assay, the ability to scavenge the stable free radical DPPH is measured by decrease in the absorbance at 517 nm. Each compound was dissolved in methanol (10mg/10 mL) and it was used as stock solution. From the stock solutions, 1mL of each compound solution with different concentrations (0.25 µg–1.00 µg) was added to the 3mL of methanolic DPPH (0.004%) solution. After 30 min, the absorbance of the test compounds was taken at 517 nm using UV-VIS spectrophotometer. BHT was used as standard, DPPH solution was used as control without the test compounds, and methanol was used as blank. The percentage of scavenging activity of DPPH free radical was measured by using the following formula:

\[
\text{Scavenging activity (\%)} = \left(1 - \frac{A_i}{A_0}\right) \times 100
\]  

Where \(A_0\) is the absorbance of the control and \(A_i\) is the absorbance of the sample.
2.7. Cytotoxic Activity.

The human breast carcinoma cell line (MCF-7), human colon carcinoma cell line (COLO 205), and murine microphage cell line (Raw 264.7) were obtained from the National Centre for Cell Science (NCCS), Pune, and grown in Dulbecco’s Modified Eagles Medium (DMEM) containing 10% fetal bovine serum (FBS), amphotericin (3 µg/mL), gentamycin (400 µg/mL), streptomycin (250 µg/mL), and penicillin (250 units/mL) in a carbon dioxide incubator at 5% CO₂. About 700 cells/well were seeded in 96-well plate using culture medium; the viability was tested using trypan blue dye with help of haemocytometer and 95% of viability was confirmed. After 24 h, the new medium with compounds in the concentration of 100, 10, and 1 µg/mL were added at respective wells and kept in incubation for 48 h. After incubation MTT assay was performed.

2.7.1. MTT Assay.

After 48 h of the drug treatment the medium was changed again for all groups and 10 µL of MTT (5mg/mL stock solution) was added and the plates were incubated for an additional 4 h. The medium was discarded and the formazan blue, which was formed in the cells, was dissolved with 50 µL of DMSO. The optical density was measured on microplate spectrophotometer at a wavelength of 570 nm. The percentage of cell inhibition was calculated by using the following formula [15]:

\[
\% \text{ Growth inhibition} = 100 - \left( \frac{A_o}{A_i} \right) \times 100
\]

Where \(A_i\) is the absorbance of the sample and \(A_o\) is the absorbance of the control. IC₅₀ values were determined using Graph Pad Prism software.
2.8. DNA Cleavage Activity.

The DNA cleavage activity of metal complexes was monitored by agarose gel electrophoresis. pBR322 plasmid was cultured, isolated, and used as DNA for the experiment. Test samples (1mg/mL) were prepared in DMF. 25 μg of the test samples was added to the isolated plasmid and incubated for 2 h at 37°C. After incubation, 30 μL of plasmid DNA sample mixed with bromophenol blue dye (1:1) was loaded into the electrophoresis chamber wells along with the control DNA, 5M FeSO₄ (treated with DNA), and standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0, 0.5M EDTA/1 L). Finally, it was loaded on to an agarose gel and electrophoresed at 50V constant voltage up to 30min. After the run, gel was removed and stained with 10.01 μg/mL ethidium bromide and image was taken in Versadoc (Biorad) imaging system. The results were compared with standard DNA marker. The same procedure was followed in the presence of H₂O₂ also.

3. Results and Discussion

All the Ni(II) complexes were colored, stable, and nonhygroscopic in nature. The complexes are insoluble in common organic solvents but soluble in DMF and DMSO. The elemental analysis showed that the complexes have 1:2 stoichiometry of the type [M(L̄1–4)₂]Cl₂, where L stands for singly deprotonated ligands. Molar conductance of the complexes was measured in DMF. The conductance values, which are presented in the Table 1, indicate the no electrolytic nature of the complexes [16].

3.1. Determination of the Metal Content of the Complexes.

Known amount (0.150 g) of complexes was decomposed with concentrated nitric acid. This process was repeated till the organic part of the complexes got completely lost. The excess nitric acid was expelled by evaporation with concentrated sulphuric acid. The Ni(II) and
Zn(II) contents of the complexes were determined as per the procedure available in the literature [17].

**Spectral Data:**

A signal at 154.9 ppm in the $^{13}$C-NMR spectrum of compound L indicated the formation of a 1,5-disubstituted tetrazole and the presence of a signal at 2220 cm$^{-1}$ in the IR spectrum indicated an azide bond (N-N or N=N band) in fig. 3 [18]. The $^1$H NMR spectrum of L, showed the expected signals for the pyridine ring, while the NH proton on the tetrazole has appeared as a broad signal at 7.1 ppm. Ligand (L) on treatment with N,N-Dimethyl-[2-(5-pyridin-2-yl-tetrazol-1-yl)-ethyl]-amine. HCl and 2-chloroethanol in basic medium afford regio isomers L$^1$, L$^2$, L$^3$ and L$^4$ by alkylation at either the 1-N or 2-N positions (Scheme 1). The structures for the isomers are readily assigned by their $^{13}$C NMR and $^1$H-NMR spectra.

The chemical shift values for the quaternary Carbon of tetrazole ring appeared in range ~150.1 - 154.7 ppm in the 1-N- in fig: 1 and 2-N-isomers in fig:2 respectively. The 1-N isomer gave the signal at 150.45 (L$^1$) and 152.15 (L$^2$) ppm respectively; while the 2-N isomer gave the signal at 151.35(L$^3$) and 154.77(L$^4$) ppm respectively. The $^1$H NMR spectra of L$^1$-L$^4$ showed separately four signals corresponding to pyridyl protons and two triplets for the methylene groups. A singlet is observed in the spectra of L$^1$ and L$^3$ at 2.29 and 2.27 ppm which indicated the presence of six protons of –N(CH$_3$)$_2$ groups respectively. The methylene protons of L$^1$-L$^4$ beside the tetrazole ring appeared at 5.12, 5.06, 5.08 and 4.87 ppm respectively. Presence of – N(CH$_3$)$_2$ in L$^2$ and L$^4$ is confirmed by I.R spectra with broad peak at 1190-1058 cm$^{-1}$.

I.R spectra of ligands (L$^2$ and L$^4$) show a broad band in range 1190-1058 cm$^{-1}$ corresponding to – N(CH$_3$)$_2$, and peaks at 1650-1500 cm$^{-1}$. Two similar peaks around 1150-900 cm$^{-1}$ corresponds to tetrazole group [19]. The formation of coordination bonds between tetrazole ring and Ni(II) and Zn(II) is confirmed by observing the IR frequencies of tetrazole.
ring. The ligands $L^1$-$L^4$ showed characteristic absorption bands (IR) at 1630-1570 cm$^{-1}$ which are shifted to lower frequencies in all Ni(II) and Zn(II) complexes. Additional peaks around 1340-1200 cm$^{-1}$ and 800-600 cm$^{-1}$ are appeared due to the coordination of pyridine ring with Ni(II) and Zn(II) atom.

The ligands ($L^1$-$L^4$) are treated with NiCl$_2$.2H$_2$O and ZnCl$_2$.2H$_2$O salt, in methanol at reflux temperature under N$_2$ atmosphere for 2-3 h. All reactions were carried out using a 1:2 metal: ligand stoichiometry ratio to give corresponding complexes (1-4) (Scheme 2). Physical properties of Nickel and Zinc are shown in the synthesis. Elemental analysis of the obtained complexes showed that these (1-4) are in 1:2 compositions. All Nickel complexes have magnetic moments value ranging 3.2 BM, slightly higher than the spin only values (2.82 $\mu_{\text{eff}}$) expected for a d$^8$ system with one unpaired electron [20] in Ni(II) complexes. ESI$^+$-Mass spectra absorption peak at m/z: 219 in fig:4

**Electronic Spectra and Magnetic Moments:**

The electronic absorption spectra of the isomeric pyridyl tetrazole metal complexes in solid state were recorded at room temperature and the band positions of the absorption maxima, band assignments, ligand field parameters, and magnetic moment values are listed in Table 1. The electronic spectra of Ni(II) complexes displayed three absorption bands in the range 8000–9000, 14000–16000, and 20000–24000 cm$^{-1}$. Thus, these bands may be assigned to the three spin allowed transitions $3A_2g$ (F)$\rightarrow$ $3T_2g$ (F) ($\nu_1$), $3A_2g$ (F)$\rightarrow$ $3T_1g$ (F) ($\nu_2$), and $3A_2g$ (F)$\rightarrow$ $3T_1g(P)$ ($\nu_3$), respectively, characteristic of octahedral geometry. The values of transition ratio [$\nu_2/\nu_1$] and $\beta$ lie in the range of 1.70–1.80 and 0.89–0.95, respectively, providing further evidence for octahedral geometry of Ni(II) complexes [21]. The $\beta$ values for the complexes are lower than the free ion value, there by indicating orbital overlap and delocalisation of d-orbitals. The $\beta$-values obtained are less than unity suggesting the covalent character of the
metal-ligand bonds. All Ni(II) complexes are paramagnetic and the magnetic movement values at room temperature are in the range of 2.91–3.25 B.M which is well agreed with the reported octahedral Ni(II) complexes [22]. All Zn(II) complexes showed two bands around 25000 and 30000 cm\(^{-1}\) and are attributed to the n→π\(^*\) and π→π\(^*\) transitions, respectively. Zn(II) complexes that are in d\(^{10}\) configuration are diamagnetic and do not show any d-d transitions.

3.6.5. DNA cleavage studies

The interaction of plasmid pBR322 DNA with Ni(II) and Zn(II) complexes was studied using gel electrophoresis in the presence and absence of oxidizing agent H\(_2\)O\(_2\). DNA cleavage was achieved by monitoring the gel electrophoresis for naturally occurring, covalently closed circular form (Form I) transition to the nicked circular (Form II) and linear forms (Form III).

When circular plasmid DNA is subjected to electrophoresis, relatively fast migration will be observed for the super coil form (Form I), slower migration will be observed for nicked circular form (Form II) and linear form occurred between the super coiled and nicked circular forms (Li et al., 2011; Kashanian et al., 2012). The gel electrophoresis pictures are shown in Fig. 5. In the absence of H\(_2\)O\(_2\), control DNA (In Fig. 5a & 5b. Lane control) does not show any activity. FeSO\(_4\) was used as standard, disappearance of bands was observed in all the complexes except [Ni(L\(^3\))\(_2\)]Cl\(_2\) and [Zn(L\(^3\))\(_2\)]; [Zn(L\(^4\))\(_2\)]Cl\(_2\) complexes indicate the complete DNA cleavage activity. In the case of [Ni(L\(^3\))\(_2\)]Cl\(_2\), [Zn(L\(^3\))\(_2\)], and [Zn(L\(^4\))\(_2\)]Cl\(_2\) complexes a decrease in the intensity of bands was observed compared to the control. This is probably due to the partial cleavage of the DNA. The DNA cleavage activity of the complexes in the presence of H\(_2\)O\(_2\) may be due to the reaction of hydroxy radicals with DNA. The general oxidative mechanisms of the DNA cleavage studies were reported by several research groups [23–25]. Many literature report infer that the compound was to cleave the DNA; it can be
concluded that the compound inhibits the growth of the pathogenic organism by cleaving the genome [26].

**Figure 5:** Gel electrophoresis photograph of metal complexes. (a) Gel electrophoresis photograph showing the effects of metal complexes on Pla322 DNA: lane 1, DNA + [Ni(L1)2]; lane 2, DNA + [Ni(L2)2]Cl2; lane 3, DNA + [Ni(L3)2]Cl2; lane 4, DNA + [Ni(L4)2]Cl2; lane 5, DNA + [Zn(L1)2]Cl2; lane 6, DNA + [Zn(L2)2]; lane 7, DNA + [Zn(L3)2]; lane 8, DNA + [Zn(L4)2]Cl2; lane 9, DNA + FeSO4; lane C, DNA alone. (b) Gel electrophoresis photograph showing the effects of metal complexes on pBR322-DNA in the presence of H2O2: lane 1, DNA + [Ni(L1)2] + H2O2; lane 2, DNA + [Ni(L2)2]Cl2 + H2O2; lane 3, DNA + [Ni(L3)2]Cl2 + H2O2; lane 4, DNA + [Ni(L4)2] + H2O2; lane 5, DNA + [Zn(L1)2]Cl2 + H2O2; lane 6, DNA + [Zn(L2)2] + H2O2; lane 7, DNA + [Zn(L3)2] + H2O2; lane 8, DNA + [Zn(L4)2]Cl2 + H2O2; lane 9, DNA + FeSO4 + H2O2; lane C2, DNA + H2O2; lane C1, DNA alone.
Conclusions

Ni(II) and Zn(II) complexes have been synthesized using bidentate isomeric pyridyl tetrazole ligands and characterized by various analytical and spectral data. Based on the electronic spectra, magnetic moment, and elemental analysis data, octahedral geometry was proposed for Ni(II) and Zn(II) complexes. The nematicidal activity of metal complexes revealed that [Ni(L₃)₂]Cl₂ and [Zn(L₃)₂]Cl₂ complexes showed moderate activity. [Zn(L₄)₂]Cl₂ complex exhibited greater antioxidant activity compared to the remaining metal complexes. All metal complexes exhibited considerable cytotoxic activity against Raw, MCF-7, and COLO 205 cell lines. The DNA cleavage studies of metal complexes showed more prominent activity in the presence of H₂O₂ compared to that in the absence of H₂O₂.
Table 1: Elemental analysis and physical properties of Ni(II) and Zn(II) complexes.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Molecular formula</th>
<th>Colour</th>
<th>% Yield (%)</th>
<th>% Found (cald.)</th>
<th>Molar conductivity (Ohm$^{-1}$ cm$^2$ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni(L$^1$)Cl$_2$</td>
<td>C$<em>{16}$H$</em>{14}$Cl$<em>2$N$</em>{10}$Ni</td>
<td>Dark Green solid</td>
<td>40.38</td>
<td>2.96</td>
<td>29.43</td>
</tr>
<tr>
<td>Ni(L$^2$)Cl$_2$</td>
<td>C$<em>{22}$H$</em>{32}$Cl$<em>2$N$</em>{12}$Ni</td>
<td>Pale Green precipitate</td>
<td>44.47</td>
<td>5.43</td>
<td>28.29</td>
</tr>
<tr>
<td>Ni(L$^3$)Cl$_2$</td>
<td>C$<em>{16}$H$</em>{14}$Cl$<em>2$N$</em>{10}$Ni</td>
<td>Pale Green crystals</td>
<td>40.38</td>
<td>2.96</td>
<td>29.43</td>
</tr>
<tr>
<td>Ni(L$^4$)Cl$_2$</td>
<td>C$<em>{22}$H$</em>{32}$Cl$<em>2$N$</em>{12}$Ni</td>
<td>Pale Green precipitate</td>
<td>44.47</td>
<td>5.43</td>
<td>28.29</td>
</tr>
<tr>
<td>Zn(L$^1$)Cl$_2$</td>
<td>C$<em>{16}$H$</em>{14}$Cl$<em>2$N$</em>{10}$Zn</td>
<td>Waxy cream solid</td>
<td>39.81</td>
<td>2.92</td>
<td>29.02</td>
</tr>
<tr>
<td>Zn(L$^2$)Cl$_2$</td>
<td>C$<em>{22}$H$</em>{32}$Cl$<em>2$N$</em>{12}$Zn</td>
<td>Cream solid</td>
<td>43.97</td>
<td>5.37</td>
<td>27.97</td>
</tr>
<tr>
<td>Zn(L$^3$)Cl$_2$</td>
<td>C$<em>{16}$H$</em>{14}$Cl$<em>2$N$</em>{10}$Zn</td>
<td>Waxy orange solid</td>
<td>39.81</td>
<td>2.92</td>
<td>29.02</td>
</tr>
<tr>
<td>Zn(L$^4$)Cl$_2$</td>
<td>C$<em>{22}$H$</em>{32}$Cl$<em>2$N$</em>{12}$Zn</td>
<td>Cream solid</td>
<td>43.97</td>
<td>5.37</td>
<td>27.97</td>
</tr>
</tbody>
</table>
Table 2. Electronic, Magnetic and Ligand Field Parameters of the Pyridyl-Tetrazole Ni(II) Complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Absorption maxima (cm(^{-1}))</th>
<th>Tentative assignments</th>
<th>Magnetic moment (B.M)</th>
<th>(\nu_2/\nu_1)</th>
<th>10 D(q) (cm(^{-1}))</th>
<th>B (cm(^{-1}))</th>
<th>(\beta)</th>
<th>LFSE (kJ mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{Ni}(\text{L}_1^1)]_2\text{Cl}_2)</td>
<td>8532, 15200, 24900</td>
<td>3 A2g(F) → 3 T2g(F) (v1) 3 A2g(F) → 3 T1g(F) (v2) 3 A2g(F) → 3 T1g(P) (v3)</td>
<td>3.12</td>
<td>1.78</td>
<td>8532</td>
<td>966</td>
<td>0.92</td>
<td>122.50</td>
</tr>
<tr>
<td>([\text{Ni}(\text{L}_1^2)]_2\text{Cl}_2)</td>
<td>8787, 16000, 24390</td>
<td>3 A2g(F) → 3 T2g(F) (v1) 3 A2g(F) → 3 T1g(F) (v2) 3 A2g(F) → 3 T1g(P) (v3)</td>
<td>3.18</td>
<td>1.82</td>
<td>8787</td>
<td>935</td>
<td>0.89</td>
<td>126.19</td>
</tr>
<tr>
<td>([\text{Ni}(\text{L}_1^3)]_2\text{Cl}_2)</td>
<td>8313, 14705, 24390</td>
<td>3 A2g(F) → 3 T2g(F) (v1) 3 A2g(F) → 3 T1g(F) (v2) 3 A2g(F) → 3 T1g(P) (v3)</td>
<td>3.25</td>
<td>1.76</td>
<td>8313</td>
<td>943</td>
<td>0.90</td>
<td>119.40</td>
</tr>
<tr>
<td>([\text{Ni}(\text{L}_1^4)]_2\text{Cl}_2)</td>
<td>8628, 16313, 24570</td>
<td>3 A2g(F) → 3 T2g(F) (v1) 3 A2g(F) → 3 T1g(F) (v2) 3 A2g(F) → 3 T1g(P) (v3)</td>
<td>2.91</td>
<td>1.89</td>
<td>8628</td>
<td>999</td>
<td>0.95</td>
<td>123.92</td>
</tr>
</tbody>
</table>

Table 3: IC\(_{50}\) values (µg/mL) of DPPH radical scavenging activity of ligands and their metal complexes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC(_{50}) (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL(_1^1)</td>
<td>1.28</td>
</tr>
<tr>
<td>HL(_1^2)</td>
<td>0.43</td>
</tr>
<tr>
<td>([\text{Ni}(\text{L}_1^1)]_2\text{Cl}_2)</td>
<td>1.22</td>
</tr>
<tr>
<td>([\text{Ni}(\text{L}_1^3)]_2\text{Cl}_2)</td>
<td>1.10</td>
</tr>
<tr>
<td>([\text{Zn}(\text{L}_1^1)]_2\text{Cl}_2)</td>
<td>0.72</td>
</tr>
<tr>
<td>BHT</td>
<td>0.75</td>
</tr>
</tbody>
</table>
Table 4: IC$_{50}$ values ($\mu$g/mL) of cytotoxic activity of ligands and their metal complexes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Raw</th>
<th>MCF-7</th>
<th>COLO 205</th>
</tr>
</thead>
<tbody>
<tr>
<td>L$^1$</td>
<td>46.6</td>
<td>24.6</td>
<td>20.2</td>
</tr>
<tr>
<td>L$^2$</td>
<td>56.2</td>
<td>34.0</td>
<td>39.4</td>
</tr>
<tr>
<td>L$^3$</td>
<td>52.6</td>
<td>29.1</td>
<td>15.3</td>
</tr>
<tr>
<td>L$^4$</td>
<td>47.0</td>
<td>30.2</td>
<td>68.2</td>
</tr>
<tr>
<td>Ni(L$^1$)$_2$Cl$_2$</td>
<td>223.8</td>
<td>67.2</td>
<td>54.6</td>
</tr>
<tr>
<td>Zn(L$^1$)$_2$Cl$_2$</td>
<td>44.2</td>
<td>40.1</td>
<td>54.8</td>
</tr>
<tr>
<td>Ni(L$^2$)$_2$Cl$_2$</td>
<td>36.4</td>
<td>26.6</td>
<td>60.9</td>
</tr>
<tr>
<td>Zn(L$^2$)$_2$Cl$_2$</td>
<td>33.2</td>
<td>23.2</td>
<td>42.5</td>
</tr>
<tr>
<td>Ni(L$^3$)$_2$Cl$_2$</td>
<td>54.2</td>
<td>36.8</td>
<td>71.2</td>
</tr>
<tr>
<td>Zn(L$^3$)$_2$Cl$_2$</td>
<td>33.2</td>
<td>22.6</td>
<td>42.1</td>
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<tr>
<td>Ni(L$^4$)$_2$Cl$_2$</td>
<td>58.5</td>
<td>35.8</td>
<td>49.8</td>
</tr>
<tr>
<td>Zn(L$^4$)$_2$Cl$_2$</td>
<td>44.6</td>
<td>39.4</td>
<td>53.6</td>
</tr>
</tbody>
</table>

References


1. There some description of IR, Ms and HNMR, but there are no pictures of these test.

Fig 1 $^1$H-NMR spectra of 2-[5-(pyridin-2-yl)-1H-tetrazol-1-yl]propyl-N,N-dimethylamine($L_1$) in CDCl$_3$ solvent.

Fig 2 $^1$H-NMR spectra of 2-[5-(pyridin-2-yl)-1H-tetrazol-2-yl]propyl-N,N-dimethylamine($L_1$) in CDCl$_3$ solvent
Fig 3: FT-IR spectra of 2-[5-(pyridin-2-yl)-1H-tetrazol-1-yl]propyl-N,N-dimethylamine($L^1$) in KBr.

Fig 4. ESI$^+$-Mass spectra of 2-[5-(pyridin-2-yl)-1H-tetrazol-1-yl]propyl-N,N-dimethylamine($L^1$)