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Article

Mixed ligand Copper(II) complexes of polyazole ligands with pendant arms amide and hydrazide – DNA binding activity

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ABSTRACT

New pyridyl tetrazole ligands 2-(5-(pyridin-2-yl)-1H-tetrazol-1-yl)acetamide (**L1**), 2-(5-(pyridin-2-yl)-2H-tetrazol-2-yl)acetamide (**L2**), 2-(5-(pyridin-2-yl)-1H-tetrazol-1-yl)acetohydrazide (**L3),** and 2-(5-(pyridin-2-yl)-2H-tetrazol-2-yl)acetohydrazide **(L4**) have been prepared. These ligands and phenanthroline have been coordinated with $CuCl_2.2H_2O$ to furnish corresponding mixed complexes **[Cu(L1)(Phen)]-[Cu(L4)(Phen)]**. Electron paramagnetic resonance spectra of all the copper complexes are characteristic of square planar geometry, with nuclear hyperfine spin 3/2. DNA binding studies were carried using UV-Vis absorption spectroscopy. Viscosity and thermal denature studies revealed that each of these complexes is avid binders of calf thymus DNA.

Key words: Pyridyl-tetrazole, Pendant arm, Mixed ligand copper complexes, DNA binding properties

1. INTRODUCTION

Tetrazoles exhibit various biological activities and act as a pharmacophore for the carboxylate group [1]. Recently, there is a growing demand for the use of pyridyl tetrazoles to prepare metal–organic frameworks which have attracted remarkable attention in the past decade as a result of their amazing structural topographies as well as their excellent properties and applications, including storage of gases, catalysis, drug delivery, magnetism, and luminescence [2]. The metal complexes of tetrazoles find a wide range of biochemical and pharmaceutical applications with respect to high physiological activity and low toxicity of tetrazoles [3]. Tetrazoles are important tools in synthetic organic chemistry and also used as precursors of carbenes in flash pyrolysis [4]. The pharmacological applications of tetrazoles with glycosidase inhibitory, antihypertensive, anti-inflammatory, antibacterial, antifungal, analgesic, antinociceptive, anticancer, anticonvulsant, antidiabetic, antiulcer, and antitubercular activities are reported [5]. Tetrazoles play a significant role in coordination chemistry as ligands, metabolically stable surrogate for a carboxylic acid group in medical chemistry, and as special explosives in materials science [6]. Tetrazole closely resembles the carboxylic group in acidic characteristics and is metabolically stable [7]. Furthermore, the applications of 5-substituted-1*H*-tetrazoles as lipophilic spacers are reported [8]. In addition, pyridines are associated with diverse biological activities [9]. In continuation of our ongoing research [10], we altered the pendant arms with acetamide and acetohydrazide groups and prepared corresponding pyridyl tetrazole copper(II) complexes.

2. EXPERIMENT

2.1. Materials and Measurements

Picolinonitrile was purchased from Sigma-Aldrich. 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. Hydrazine hydrate is a hazardous compound, and MSDS data sheet was referred before using it. All melting points were obtained using Elico instrument, India (Model MP96), and are uncorrected. Mass spectra were obtained on a Pexciex API 2000 eV spectrometer, Q1MSQ1/autoinjection mass spectra. ^1H and ^{13}C NMR spectra were recorded on a Bruker TopSpin Instrument. Infrared (IR) spectra were recorded using Alpha T OPUS instrument. Elemental analysis (% of C, H, and N) was carried out using a PerkinElmer 2400 elemental analyzer. Magnetic moments were determined in the polycrystalline state on a PAR model-155 vibrating sample magnetometer operating at a field strength of 2–8 kG. High purity Ni metal (saturation moment 55 emug¢1) was used as standard. Electron paramagnetic resonance (EPR) spectra were recorded on Varian E-122 X-band spectrophotometers at liquid nitrogen temperature in DMF.

2.2. General Procedure for the Synthesis of 3 and 4 Ligands

To a solution of tetrazole **2** (1 g, 6.8 mmol) in DMF (15 ml), ethylbromo acetate (0.75 ml, 6.8 mmol) was added. The mixture was allowed to stir for 8 h at 70°C. The reaction mixture was diluted with ethyl acetate (50 ml), and the organic layer was washed with saturated $NaHCO_3$ (50 ml) and washed successively with water (40 ml 3 ml) followed by brine solution (40 ml). The organic layer was dried over $MgSO_4$, filtered, and the solvent was evaporated under reduced pressure to afford a brown-colored gummy liquid, which was purified

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by column chromatography using 17% EtOAc in hexane (V/V) to afford the esters **3** and **4**.

Ethyl 2-(5-(pyridin-2-yl)-1H-tetrazol-1-yl)acetate (**3**) [2] yellowish solid; yield: 0.45 g (28%). Yellow solid. M.p.54–57°C. Anal. Calc. for $C_{10}H_{11}N_5O_2$ (233.23): % of C, 51.50; H, 4.75; O, 13.72; N, 30.03. Found: % of C, 51.48; H, 4.74; O, 13.69; N, 30.01. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.66 (d, 1H, J=3.3 Hz), 8.43 (d, 1H, *J*=8.1 Hz), 7.92 (dt, 1H, J=7.8, 1.8Hz), 7.48–7.41 (m, 1H), 5.75 (s, 2H), 4.19 (q, 2H, 6.9 Hz), 1.21 (t, 3H, *J*=6.9 Hz) ppm.

Ethyl 2-(5-(pyridin-2-yl)-2H-tetrazol-2-yl)acetate (**4**) [2] yellowish solid yield: 0.80 g (50 %). White solid. M.p. 94–98°C. Anal. Calc. for $C_{10}H_{11}N_5O_2$ (233.23): % of C, 51.50; H, 4.75; O, 13.72; N, 30.03. Found: % of C, 51.47; H, 4.71; O, 13.71; N, 29.99. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.79 (d, 1H, *J*=4.8 Hz), 8.28 (d, 1H, *J*=7.8 Hz), 7.88 (td, 1H, *J*=7.8, 1.8 Hz), 7.42 (m, 1H), 5.50 (s, 2H), 4.29 (q, 2H, *J*=7.2 Hz), 1.29 (t, 3H, *J*=7.2 Hz) ppm.

2.3. General Procedure for the Synthesis of 5 and 6 Ligands

To the methanolic (21 ml) solution of ester **3** or **4** (0.5 g, 2.14 mmol), 2 ml of aqueous NaOH (1N) was added and the resulting solution was stirred for 6 h at room temperature, and the formation of a white precipitate in the reaction mixture was observed. The reaction was quenched with 3–4 drops of acetic acid and 10 ml of ethyl acetate was added. Then, the mixture was filtered and the residue was washed with ethyl acetate (20 ml), and the colorless residue was collected and dried in air.

2-(5-(Pyridin-2-yl)-1H-tetrazole-1-yl)acetic acid (**5**): Color less solid; yield: 400 mg (91%). M.p.284-287°C. Anal. Calc. for $C_8H_7N_5O_2$ (205.17): % of C, 46.83; H, 3.44; O, 15.60; N, 34.13. Found: % of C, 46.80; H, 3.40; O, 15.57; N, 34.12. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.72 (t, 1H, *J*=4.8,3.9 Hz), 8.12 (d, 1H, *J*=7.8 Hz), 8.03 (m, 1H), 7.58 (m, 1H), 5.45 (s, 2H) ppm. ^{13}C NMR ($CDCl_3$, 65 MHz): δ 172.5, 153.1, 149.9, 143.2, 138.4, 126.3, 124.4, 52.9. Electrospray ionization-mass spectrometry (ESI-MS): m/z 204 (M-1).

2-(5-(Pyridin-2-yl)-2H-tetrazole-2-yl)acetic acid (**6**): Color less solid; Yield: 0.38 g (86%). M.p. 277–279°C. Anal. Calc. for $C_8H_7N_5O_2$ (205.17): % of C, 46.83; H, 3.44; O, 15.60; N, 34.13. Found: % of C, 46.79; H, 3.42; O, 15.55; N, 34.10. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.73 (d, 1H, *J*=4.5 Hz), 8.11 (d, 1H, *J*=8.1 Hz), 7.98 (td, 1H, *J*=7.5, 1.5 Hz), 7.52 (m, 1H), 4.97 (s, 2H) ppm. ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 172.19, 164.49, 150.15, 145.47, 139.25, 126.59, 123.58, 56.71 ppm. ESI-MS: m/z 204 (M-1).

2.4. General Procedure for the Synthesis of L1 and L2

To a solution of acid **5 or 6** (0.5 g, 2.44 mmol) in dioxane (30 ml), a mixture of BOC anhydride (0.85 ml, 3.7 mmol) and pyridine (0.24 ml, 2.93 mmol) was added at r.t. The mixture was stirred over a period of 0.5 h. Then, ammonium carbonate (281 mg, 2.93 mmol) was added and the resulting solution was stirred for 8 h. The reaction mixture was filtered, and the filtrate was extracted with ethyl acetate (30 ml). The organic layer was washed with water (30 ml × 3 ml) followed by brine solution to remove any impurities in the product. The organic layer was dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure to afford a crude product which was triturated with diethyl ether to furnish colorless solid.

Ethyl 2-(5-(pyridin-2-yl)-1H-tetrazol-1-yl)acetamide (**L1**): Colorless solid; yield: 0.25 g (50%). M.p. 97-99°C. Anal. Calc. for $C_8H_8N_6O$ (204.19): % of C, 47.06; H, 3.95; O, 7.84; N, 41.16. Found: % of C, 47.03; H, 3.92; O, 7.83; N, 41.14. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.73 (t, 1H, *J*=4.2, 3.3 Hz), 8.16 (d, 1H, *J*=7.8 Hz), 8.05 (m, 1H), 8.01 (br. s, 1H), 7.59 (m, 2H), 6.14 (br. s, 1H), 6.13 (s, 2H) ppm. ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 172.6, 153.3, 149.4, 143.6, 138.4, 126.3, 124.6, 53.1 ppm. ESI-MS: m/z 205 (M+1).

Ethyl 2-(5-(pyridin-2-yl)-2H-tetrazol-2-yl)acetamide (**L2**): Colorless solid; yield: 0.36 g, (72%). M.p. 98–103°C. Anal. Calc. for $C_8H_8N_6O$ (204.19): % of C, 47.06; H, 3.95; O, 7.84; N, 41.16. Found: % of C, 47.03; H, 3.92; O, 7.83; N, 41.14. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.75 (t, 1H, *J*=4.2, 3.3 Hz), 8.15 (d, 1H, *J*=7.8 Hz), 8.02 (td, 1H, *J*=7.5, 1.5 Hz), 7.90 (br-s, 1H), 7.56 (m, 2H), 6.16 (br-s, 1H), 5.51 (s, 2H) ppm. ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 172.2, 164.5, 150.2, 145.5, 139.3, 126.6, 123.6, 56.7 ppm; ESI-MS: m/z 205 (M+1).

2.5. General Procedure for the Synthesis of L3 and L4

To the ethanolic solution (10 ml) of ester **3** or **4** (0.5 g, 2.14 mmol), hydrazine hydrate (0.2 ml, 4.28 mmol) was added and the resulting solution was stirred for 10 h at 80°C. The solvent was removed under reduced pressure, and the crude material was triturated with diethyl ether to afford colorless solid.

2-(5-(Pyridin-2-yl)-1H-tetrazol-1-yl)acetohydrazide (**L3**): Colorless solid; yield: 0.32 g (68%). M.p. 185–187°C. Anal. Calc. for $C_8H_9N_7O$ (219.20): % of C, 43.83; H, 4.14; O, 7.30; N, 44.73. Found: % of C, 43.80; H, 4.12; O, 7.25; N, 44.71. ^{1}H NMR (DMSO-D6, 300 MHz): δ 9.48 (br-s, 1H-NH), 8.72 (d, 1H, *J*=4.5 Hz), 8.30 (d, 1H, *J*=12.9 Hz), 8.08 (dt, 1H, *J*=7.8, 1.8 Hz), 7.62 (dd, 1H, *J*=4.8, 1.8 Hz), 5.64 (s, 2H), 4.29 (br-s, 2H-NH2) ppm. ^{13}C-NMR (DMSO-D6, 75 MHz): δ 166.7, 152.6, 149.7, 144.2, 138.2, 125.9, 123.7, 48.6 ppm; ESI-MS: m/z 220 (M+1).

2-(5-(Pyridin-2-yl)-2H-tetrazol-2-yl)acetohydrazide (**L4):** Colorless solid; yield: 0.32 g (68%). M.p.168–171°C. Anal. Calc. for $C_8H_9N_7O$ (219.20): % of C, 43.83; H, 4.14; O, 7.30; N, 44.73. Found: % of C, 43.80; H, 4.12; O, 7.25; N, 44.71. ^{1}H NMR (DMSO- D6, 300 MHz): δ 10.8 (br-s, 1H-NH), 8.76 (d, 1H, *J*=4.5 Hz), 8.13 (d, 1H, *J*=7.8 Hz), 8.02 (dt, 1H, *J*=7.5, 1.5 Hz), 7.57 (dd, 1H, *J*=4.8, 1.0 Hz), 5.88 (s, 2H), 3.56 (br-s, 2H-NH_2) ppm. ^{13}C-NMR (DMSO-D6, 75 MHz): δ 166.5, 164.2, 150.4, 145.6, 139.3, 126.2, 123.3, 51.4 ppm; ESI-MS: m/z 220 (M+1).

2.6. General Procedure for the Synthesis of Copper Complexes [Cu(L1)(Phen)] to [Cu(L4)(Phen)]

To a solution of **L1** to **L4** (0.488 mmol) and phenanthroline (0.488) in methanol (10 ml), $CuCl_2.2H_2O$ (0.244 mmol) was added and the resulting solution was stirred for 2 h at 70°C. Then, the reaction mixture was allowed to cool and the solvent was evaporated slowly to expect the crystals. However, after 7 days, the evaporation afforded only green color solids. The solids were triturated with diethyl ether and used without further purifications.

[Cu(L1)(Phen)]: 189 mg, 0.4 mmol; IR (KBr disc, cm^{-1}): 3445, 2938, 2027, 1636, 1575, 1400, 1316, 1294, 1084, 985,896. Anal. calc. for $C_{16}H_{16}N_{12}O_2Cu$ (472): % of C, 40.72; H, 3.42; N, 35.62; Found: % of C, 40.34; H, 3.36; N, 35.55. ESI-MS: m/z 472 (M+1).

[Cu(L2)(Phen)]: 195 mg, 0.42 mmol; IR (KBr disc, cm^{-1}): 3448, 2981, 2067, 1635, 1568, 1397, 1252, 1168, 1053, 789, $C_{16}H_{16}N_{12}O_2Cu$ (472): % of C, 40.72; H, 3.42; N, 35.62. Found: % of C, 40.28; H, 3.40; N, 35.52. ESI-MS: m/z 472 (M+1).

[Cu(L3)(Phen)]: 210 mg, 0.42 mmol; IR (KBr disc, cm^{-1}): 3413, 3254, 2033, 1763, 1637, 1618, 1455, 1309, 1294, 1229, 1110, 1013, 788, 748. Anal. calc. for $C_{16}H_{18}N_{14}CuO_2$ (502): % of C, 38.28; H, 3.61; N, 39.07. Found: % of C, 38.10; H, 3.51; N, 39.03. ESI-MS: m/z 502 (M+1).

[Cu(L4)(Phen)]: 195 mg, 0.39 mmol; IR (KBr disc, cm^{-1}): 3453, 3254, 2042, 1753, 1628, 1592, 1438, 1395, 1313, 1170, 1113, 1013, 786, 728. Anal. calc. for $C_{16}H_{18}N_{14}CuO_2$ (502): % of C, 38.28; H, 3.61; N, 39.07. Found: % of C, 38.18; H, 3.48; N, 39.05. ESI-MS: m/z 502 (M+1).

2.7. Antioxidant activity by P-NDA (p-nitroso dimethylaniline) radical scavenging method:

To a solution containing $FeCl_3$ (0.1mM, 0.5 ml), EDTA (0.1mM, 0.5 ml), ascorbic acid (0.1mM, 0.5 ml), hydrogen peroxide (2mM, 0.5 ml), and *p*-nitroso dimethylaniline (0.01mM, 0.5 ml) in phosphate buffer (pH 7.4, 20 mM), various concentrations of the test compounds were added in distilled DMSO to produce final volume of 3 ml. Absorbance was measured at 440 nm.

$$\text{p-NDA radical scavenging activity (\%)} = \frac{[\text{Abs (sample)-Abs (standard)}]}{[\text{Abs (sample)}]} \times 100 \quad (1)$$

where Abs - Absorbance.

2.8. DNA Binding Experiments

All measurements with CT DNA were performed in buffer Tris–HCl 5 mM (pH 7.2), 50 mM NaCl. The UV absorbance ratio 260/280 was 1.8–1.9, indicating that the DNA was sufficiently free of protein [11]. The concentration of CT DNA per nucleotide was determined from the absorption intensity at 260 nm with the known value of 6600 M^{-1} cm^{-1}. The absorption titrations were performed by adding increasing amounts of CT DNA to a solution of the complex at a fixed concentration contained in a quartz cell and recording the UV-Vis spectrum after each addition. The absorption of CT DNA was subtracted by adding the same amount of DNA to a blank. The data were then fitted to equation 2 to obtain the intrinsic binding constant, K_b [12].

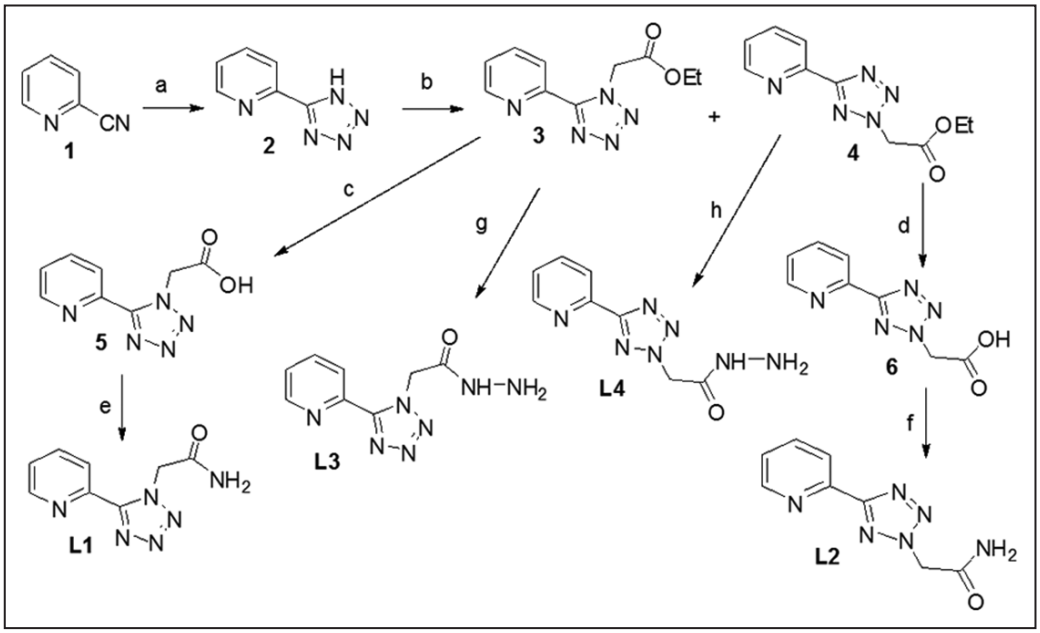
$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f) \quad (2)$$

where [DNA] is the molar concentration of CT-DNA, and ε_a, ε_b, and ε_f are apparent, free, and bound metal complex extinction coefficients, respectively. Thus, K_b is the ratio of the slope to the y-intercept.

3. RESULTS AND DISCUSSION

The synthetic route was started from picolinonitrile **1** by following the method available in the literature to form the tetrazole **2,** and the structure was confirmed by comparing the analytical data of **2** with the reported data [13,14]. Tetrazole **2** on alkylation with ethyl bromoacetate in dry DMF at 70°C afforded regioisomers **3** and **4** by alkylation at N(1) or N(2) positions of tetrazole rings, respectively (Scheme 1). The structures of the isomers are readily assigned by their ^{1}H NMR spectra. Compound **3** showed characteristic peaks at δ 5.75 (-N-CH_2-CO-), 4.19 (O-CH_2), and 1.21(-CH_3) which confirmed the formation of ethyl ester. Compound **4** showed characteristic peaks at δ 5.5 (-N-CH_2-CO-), 4.29 (O-CH_2), and 1.29 (-CH_3) which confirmed the formation of ethyl ester. The ethyl esters are further confirmed by comparing the data with reported compounds [2]. The ethyl ester derivatives **3** and **4** are hydrolyzed to acid derivatives **5** and **6,** respectively, by treating with aqueous 1N NaOH. The ^{1}H NMR spectra of **5** and **6** showed the disappearance of -OCH_2CH_3 peaks and migration of N-CH_2 peaks to upfield. The acid derivatives are further confirmed by their mass spectra which showed M-1 (negative mode) peaks at 204. The acid derivatives **5** and **6** on treatment with ammonium carbonate, BOC anhydride, and pyridine in dry 1,4-dioxane afforded the respective acetamide ligands **L1** and **L2**. The formation of **L1** is confirmed by observing –CO-NH_2 signals at δ 8.01 and 6.14 as broad singlets. The formation of **L2** is confirmed by observing –CO-NH_2 signals at δ 7.9 and 6.16 as broad singlets. Further, the mass spectra of both the ligands showed M+H peaks at 205.

The acetohydrazide ligands **L3** and **L4** were prepared by reacting the ethyl ester derivatives **3** and **4** with hydrazine hydrate in ethanol at 80°C (Scheme 1). The hydrazide **L3** was confirmed by observing its ^{1}H NMR which showed a characteristic peak of –CO-NH at δ 9.48 as broad singlet. The –NH_2 peak of hydrazide appeared as a broad singlet at δ 4.29, which confirmed the formation of hydrazide ligand **L3**. The hydrazide **L4** was confirmed by observing its ^{1}H NMR which showed a characteristic peak of –CO-NH at δ 10.8 as broad singlet. The –NH_2 peak of hydrazide appeared as broad singlet at δ 3.56, which confirmed the formation of hydrazide ligand **L4**. Further, the mass spectra of both the ligands **L3** and **L4** showed M+H peaks at 220.



Scheme 1: Synthesis of **L1-L4:** Reagents and conditions: (a) NaN_3, LiCl, NH_4Cl, DMF, reflux, 10 h; (b) ethylbromo acetate, DMF, 70°C, 8 h; (c and d) aqueous NaOH (1N), MeOH, r.t., 6 h; (e and f) BOC anhydride, pyridine, $(NH_4)_2CO_3$, dioxane, r.t., 8 h; (g and h) hyrazine hydrate, EtOH, 80°C, 10 h.

The ^{1}H NMR spectra of all the compounds **3**, **4**, **5**, **6**, **L1**, **L2**, **L3,** and **L4** showed separately four signals corresponding to pyridyl protons. The ^{13}C NMR chemical shift values for the quaternary carbon of the tetrazole rings of all the compounds **3**-**L4** appeared in the range δ ~153 ppm for the N(1)-isomers and at δ ~ 164 ppm for the N(2)-isomers.

The ligands **L1**-**L4** are treated with $CuCl_2.2H_2O$ in methanol at reflux temperature under N_2 atmosphere for 2 h. All the reactions were carried out using a 1:2 metal:ligand stoichiometry ratio to give corresponding complexes [Cu(L1)(Phen)]-[Cu(L4)(Phen)] (Scheme 2). The physical properties such as color, melting points, and magnetic moments of the complexes are shown in Table 1. The elemental analysis of the obtained complexes [**Cu(L1)(Phen)]-[Cu(L4)(Phen)]** showed that all are in 1:2 (metal: ligand) compositions. All copper complexes have magnetic moment values in the range of 1.84–1.92 BM, which is slightly higher than the spin only values (1.73 μ_{eff}) expected for a d^9 system with one unpaired electron in copper(II) complexes [15].

The ESI mass spectra of copper complexes were used to compare the stoichiometric composition of the complexes. The molecular ion peaks (M^+) of amide copper complexes [**Cu(L1)(Phen)]-[Cu(L2)(Phen)]** were observed at m/z=472 and for hydrazide copper complexes **[Cu(L3)(Phen)]-[Cu(L4)(Phen)]** were observed at 502, suggesting the stoichiometry of copper to ligand as 1:2. Elemental analysis values are in close agreement with the values obtained from ESI-mass spectra of the respective complexes.

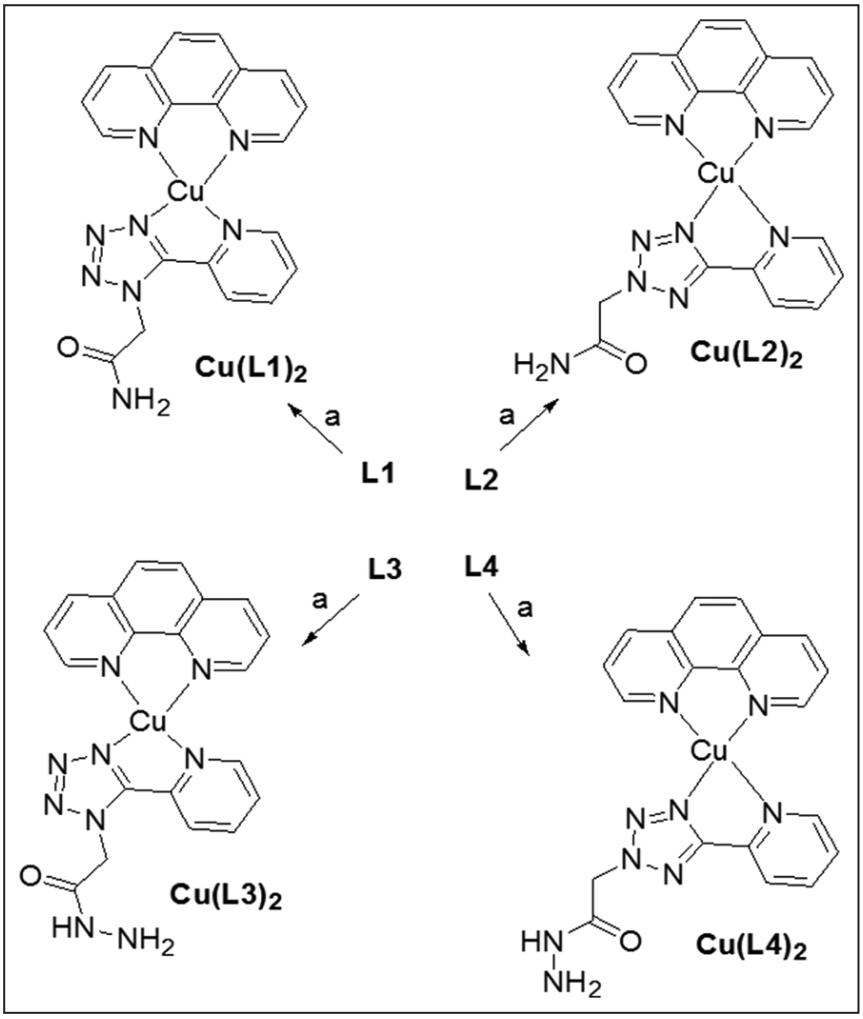
Electronic spectra of copper complexes [**[Cu(L1)(Phen)]-[Cu(L4)(Phen)]** recorded in DMF are shown in Table 2. The spectra of the

Table 1: Physical properties of pyridyl-tetrazole copper complexes **[Cu (L1)(Phen)]-[Cu (L4)(Phen)].**

S.No	Complex	Color	Melting Point (°C)	μeff (BM)
1	[Cu (L1)(Phen)]	Green	162–164	1.84
2	[Cu (L2) (Phen)]	Green	158–160	1.92
3	[Cu (L3) (Phen)]	Green	201–203	1.86
4	[Cu (L4) (Phen)]	Green	194–198	1.90

Table 2: Electronic spectral data λ_{max} (nm) of the Cu (II) complexes.

Complexes	MLCT (nm)	d-d (nm)
[Cu (L1) (Phen)]	438	655
[Cu (L2) (Phen)]	430	652
[Cu (L3) (Phen)]	437	650
[Cu (L4) (Phen)]	445	654



Scheme 2: Synthesis of [**Cu(L1)(Phen)]-[Cu(L4)(Phen)]**complexes**.** Reagents and conditions: (a) $CuCl_2.2H_2O$, methanol, 70°C, 2 h.

complexes showed bands in the region around 430–450 nm with high molar extinction coefficient in range 8300–7300 M^{-1} cm^{-1} which are assigned to the metal-to-ligand charge transfer (MLCT) transition (n – π*), while the single broadband observed in the region 655–650 nm is assigned to d-d transition. The electronic spectra of these complexes display weak d–d bands in the low intensity with molar extinction coefficients in between 130 and 95 M^{-1} cm^{-1} regions which are assigned to $^2E_g \rightarrow {}^2T_{2g}$ electronic transition, and these assignments suggest a symmetrical square planar geometry for the copper(II) complexes [16,17].

The IR spectral data of the metal complexes are given in Table 3. IR spectra of the ligands (**L1** and **L2**) show a broadband in range 3550–3100 cm^{-1} corresponding to $–NH_2$ of amide/hydrazine, and the peaks 1681 and 1685 cm^{-1} in amide ligands (**L1** and **L2**) and the peaks at 1622 and 1642 cm^{-1} in hydrazine ligands (**L3** and **L4**) are shifted to lower frequencies, suggesting the coordination of ligands to form copper complexes. The peaks at 1626–1534 cm^{-1} corresponding to pyridyl tetrazole group are shifted to lower frequency in copper(II) complexes confirming the formation of coordination of pyridyl tetrazole ring with copper [18]. Additional peaks around 1395–1250 cm^{-1} and 800–600 cm^{-1} have appeared due to the coordination of pyridine ring with copper(II) atom. All spectra of copper complexes showed a lower number of frequencies, suggesting that the complexes are symmetric in nature [19].

The solid-state EPR spectra of copper(II) complexes [**Cu(L1) (Phen)]** and **[Cu(L3) (Phen)]** were recorded in the X-band region at room temperature (25°C), and the data are summarized in Table 4. Complexes **[Cu(L1)(Phen)]** and **[Cu(L3)(Phen)]** exhibit $g_{\parallel}$ values of 2.16 and 2.18 and $g_{\perp}$ values of 2.05 and 2.06, respectively. From the observed values of complexes **[Cu(L1)(Phen)** and **Cu(L3)(Phen)**, predominantly in the dx2-y2 orbital. This is characteristic of copper(II) complexes with square planar geometry.

The geometric parameter G, which is a measure of the exchange interaction between the copper centers in the polycrystalline compound, is calculated using the equation 3.

$$G=(g_{\parallel} - 2.0023)/(g_{\perp} - 2.0023) \quad (3)$$

According to Hathaway and Tomlinson [20] if G >4.0, considerable exchange interaction is negligible because the local tetragonal axes are aligned parallel or slightly misaligned. If G <4.0, exchange is considerable and the local tetragonal axes are misaligned. The G values of the complexes **[Cu(L1)(Phen)]** and **[Cu(L3)(Phen)]** are obtained as 3.306 and 3.652, respectively, which suggested that there is no exchange interaction in the copper(II) complexes.

3.1. DNA Binding Studies

The binding interactions of copper(II) complexes with CT-DNA were monitored by UV–Vis spectroscopy. The complexes showed stability in the Tris-buffer solution and bound effectively with CT-DNA instead of Tris-buffer solution [21]. The absorption spectra of the copper complexes were compared with and without CT DNA at around 440 nm peak (Figure 1). The data of UV absorption spectra on the addition of CT-DNA and the binding constants of these complexes are given in Table 5. On addition of increasing amounts of CT-DNA, the UV–Visible absorption spectra of complexes **[Cu(L1) (Phen)]-[Cu(L4) (Phen)]** showed an increase in absorbance, exhibiting bathochromic shift (~ 0.5 nm) with hyperchromism with respect to control (0 µl DNA). The change in absorbance values with increasing amount of CT-DNA was used to evaluate the intrinsic binding constant K_b. (Table 5).

It is evident from Table 5 that all the complexes bind to DNA with high affinities and the estimated binding constants are in the range $5.0–7.2\times10^{-4}$ M^{-1}. The binding order of the complexes is **[Cu(L3) (Phen)]** > **[Cu(L4)(Phen)]** > **[Cu(L1)(Phen)]** > **[Cu(L2)(Phen)]**. The K_b value of **[Cu(L1)(Phen)]-[Cu(L4)(Phen)]** complexes is comparable with the reported values of redox active Cu(II) pyridine-based tetrazolo[1,5-a]pyrimidine complexes [22]. The intrinsic binding constant for *cis*-platin, which shows a hyperchromic shift, after subsequent addition of CT-DNA is reported as 3.2010^4 M^{-1} [23].

The binding nature of the present complexes is significant due to stacking interaction of planar pyridyl-tetrazole rings with pendant arm.

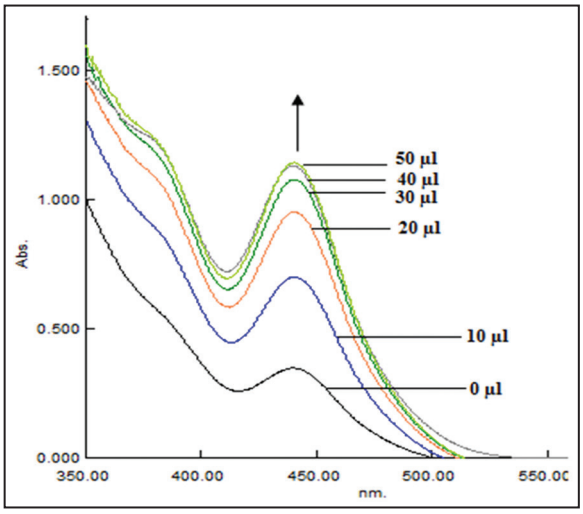


Figure 1: The UV-Vis absorption spectra of the copper complex $Cu(L3)_2$ with and without CT-DNA in Tris-buffer solution.

Table 3: Selective I.R. bands cm^{-1} of Cu (II) complexes with tentative assignments.

Vibration	L1	[Cu (L1) (Phen)]	L2	[Cu (L2) (Phen)]	L3	[Cu (L3) (Phen)]	L4	[Cu (L4) (Phen)]
Amide/	1681	1636	1685	1635	-	-	-	-
hydrazine	-	-	-	-	1622	1618	1642	1628
C=N (tetrazole)	1617	1575	1606	1568	1534	1455	1626	1592
C=C (tetrazole)	1498	1400	1486	1397	1471	1309	1508	1438
Py/M-Py	1392	1316	1334	1252	1433	1294	1443	1395

Table 4: EPR spectral assignments for complexes **[Cu (L1) (Phen)]** and **[Cu (L3)(Phen)]** at room temperature.

Complexes	$g_{\parallel}$	$g^{\perp}$	g_{av}	G
[Cu (L1) (Phen)]	2.16	2.05	2.10	3.306
[Cu (L3) (Phen)]	2.14	2.04	2.09	3.652

EPR: Electron paramagnetic resonance

Table 5: Effect of CT DNA on the absorption and binding constant of Cu (II) complexes.

Complex	λ_{max}/nm		Δλ/ nm	H%	K_b/M^{-1}
	Free	Bound			
Cu (L1) (Phen)	434.0	433.5	0.5	7.5	6.2×10^{-4}
Cu (L2) (Phen)	438.0	437.4	0.6	4.5	5.4×10^{-4}
Cu (L3) (Phen)	440.0	437.5	1.8	10.5	7.2×10^{-4}
Cu (L4) (Phen)	439.0	437.5	1.5	8.0	6.4×10^{-4}

*H%: Hyperchromism, Δλ: Bathochromic shift

Binding of complexes to CT-DNA by external contact (electrostatic or groove binding) brings about bathochromic shift with hyperchromism absorption intensity [24].

4. CONCLUSIONS

Novel pyridyl tetrazole ligands with pendant arm of acetamide and acetohydrazide and their copper complexes were synthesized and characterized. Physicochemical and spectral studies revealed that the symmetric mononuclear complexes exhibit square planar geometry. All copper complexes are avid binders of CT-DNA and showed good antioxidant properties. The highlights of the present investigations are as follows:

(i) Mixed ligand complexes of pyridyl tetrazole with amide and hydrazide pendant arms with 1,10-phenanthroline were synthesized.
(ii) The regioisomeric and symmetric nature of synthesized copper complexes is attractive feature to develop chemotherapeutic drugs.
(iii) The present study showed that the synthesized compounds can be used as a template for future development through modification and derivatization to design more potent and selective antioxidant agents.

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