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Antioxidant, Antimicrobial, Molecular Docking Studies of Novel 2,6-*bis*(1,3-Thiazol-2-yl)-4-(3,4,5-trimethoxyphenyl)pyridine and its Cu(II) and Ni(II) Complexes

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ABSTRACT

In the present study, a novel ligand 2,6-*bis*(1,3-thiazol-2-yl)-4-(3,4,5-trimethoxyphenyl)pyridine and its Cu(II) and Ni(II) complexes were synthesized. All the synthesized compounds have been characterized by ^{1}H & ^{13}C NMR, mass, UV, FT-IR and ESR spectra. The antioxidant activity of the ligand and its Cu(II) and Ni(II) complexes were evaluated by the percentage of inhibition of 1,1-diphenyl-2-picryl hydrazyl (DPPH) and compounds found to be potent antioxidants. Also, synthesized compounds showed a mild antimicrobial activity in comparison with standard drugs. Copper(II) complexes showed a good antimicrobial activity than the parent ligand and nickel(II) complex. Interestingly, ligand and its metal complexes exhibit non-toxicity as they did not cause any effect to human erythrocyte.

KEYWORDS

Antioxidant, Hemolysis, Antimicrobial activity, Molecular docking.

INTRODUCTION

Thiazole nucleus is extensively studied heterocyclic compound which possesses a spectrum of biological and pharmacological activities [1,2]. Thiazole and its derivatives play vital roles in many drugs, which possess various biological and pharmaceutical activities, such as antioxidant, antimicrobial, anti-inflammatory, antifungal, anticonvulsant, antiviral, anti-tumor, antidiabetic ,antitubercular, anticancer, *etc*. [3-6]. The discovery of compound 2,2′:6′,2″-terpyridines (tpy), have attracted widespread attention of chemists because of their excellent coordinating/complexing capacity as *N*-donor ligands towards various transition-metal and lanthanide cations. Compounds containing acetylpyridine or acetylthiazole are the starting material for the formation of a ligand by Kröhnke pyridine ligand synthesis [7-11]. So several novel ligands and metal complexes were reported on 2,2′:6′,2″-terpyridines derivatives but very few ligands and complexes of 4-(aryl)-modified-2,6-di(1,3-thiazol-2-yl) pyridine were known [12-14]. To date, only few 2,6-di(thiazol-2-yl)pyridine derivatives and their transition metal complexes have been reported [15-17].

Metal complexes of 4-aryl-modified-2,6-di(1,3-thiazol-2-yl)pyridine have the capability to form complexes with

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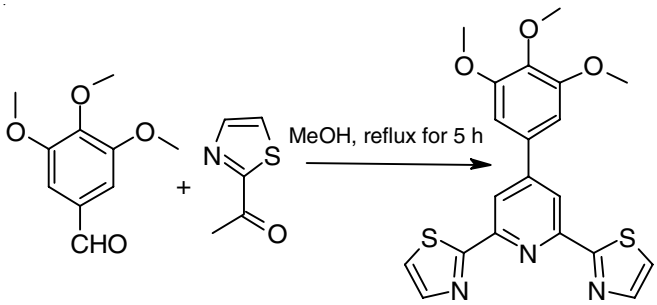
various transition metals and in view of their interesting photophysical, electronic, photonic, magnetic, reactive and structural properties, as well as promising applications in supramolecular chemistry, catalysis, molecular magnetism, molecular electronics and anti-tumour therapy. These ligands and their various transition metal complexes were studied for their photophysical and various biological and pharmacological activities like DNA binding, DNA cleaving agents, cytotoxicity, DNA interaction, anticancer activity, DFT calculations, photoluminescence and catalytic activity, antitumor, antimicrobial, or anti-HIV agents [16,18]. Numerous copper complexes have attracted significant attention due to they possess various biological activities like DNA binding, DNA cleaving agents, anticancer, antimicrobial, antioxidants, *etc*. [19].

EXPERIMENTAL

All the reagents required for the synthesis were purchased commercially from Merck and Sigma-Aldrich and used without any further purification. Solvents obtained from Spectrochem and were of analytical grades. Melting points of the compounds were recorded on a hot stage Gallen Kamp melting point apparatus. IR spectra of samples were recorded by using FTIR.8300 Shimadzu spectrophotometer in the frequency range of 4000-200 cm^{-1}. The ^{1}H & ^{13}C NMR spectra were recorded on Bruker 400 MHz spectrometer using $CDCl_3$ as the solvent and tetramethylsilane (TMS) as the internal standard. Elemental analysis was conducted by conventional methods.

Synthesis of 2,6-*bis*(1,3-thiazol-2-yl)-4-(3,4,5-trimethoxyphenyl)pyridine (SM-8b/L_9): 2-Acetylthiazole (2 mmol) in a 100 mL was added in a round-bottom flask containing MeOH (30 mL), KOH pellets (0.560 g, 4 mmol) and 2 mL of water. The mixture was stirred for 20 min and then added corresponding methanoic solution of 3,4,5-trimethoxybenzaldehyde (1 mmol) at room temperature and continued stirring for 5 h by Khronke pyridine synthesis [17]. The solid was filtered and washed with methanol and then diethyl ether. The yellow coloured solid with 85% yield obtained. The ligand obtained used for complexation without further purification (**Scheme-I**). Pale yellow solid; yield: 80%;^{1}H NMR (400 MHz, d_6-$CDCl_3$): δ 8.366 (s, 2H), 7.943-7.951 (d, 2H, *J* = 3.2 Hz), 7.482-7.490 (d, 2H, *J* = 2.8 Hz), 6.980 (s, 2H, ArH), 3.951 (s, 6H, OCH_3), 3.902 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, δ ppm): 56.443, 60.976, 104.543, 117.697, 121.978, 133.089, 144.063, 150.961, 151.447, 153.792, 168.814. Mass spectrum: M+1 peak observed at 412 (m.w. 411).

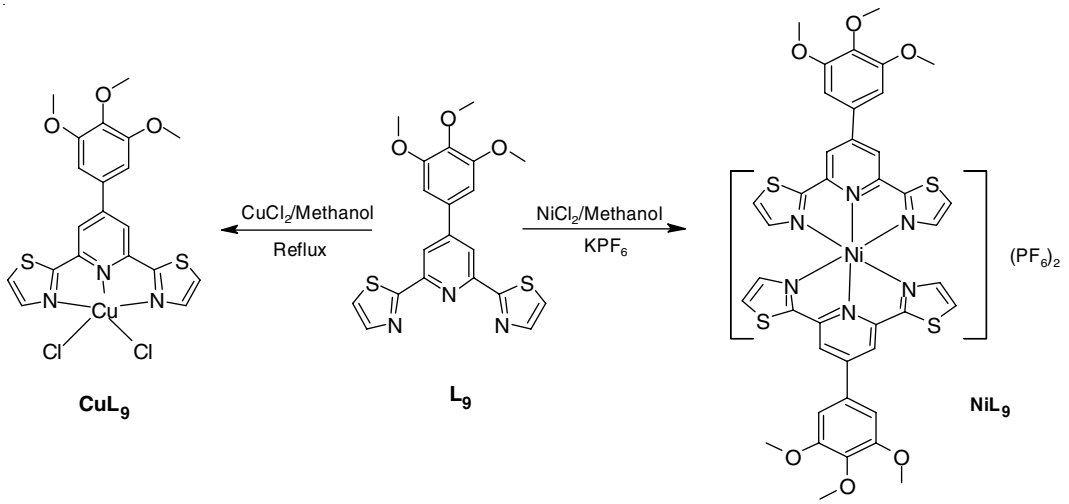


Scheme-I: 2,6-*bis*(1,3-Thiazol-2-yl)-4-(3,4,5-trimethoxyphenyl)pyridine (L_9)

Synthesis of copper(II) metal complex (M:L = 1:1): A solution of $CuCl_2{\cdot}2H_2O$ (1 mmol) dissolved in 10 mL methanol and was added to a hot methanolic solution (10 mL) of L_9. The mixture was refluxed at 50 ºC for 5 h, which resulted in the appearance of green precipitate [16]. A collected green precipitate dried with diethyl ether and recrystallized in CH_3OH-$CHCl_3$ (1:1) mixture (**Scheme-II**). The product obtained was identified by FT-IR (ATR, ν_{max}, cm^{-1}): 3018 (arom. -C-H), 1620 (C-O), 1534 (C=C), 1475 (C-N) 788 (Cu-Cl), 650 (Cu-N). UV-visible: λ_{max}: 290, 352,360 nm due to *d-d* transitions, π-π* and n-π* transitions. ESR spectra: g = 2.10753.

Synthesis of nickel(II) complex (M:L = 1:2): Nickel(II) complex was also synthesized by the same procedure as copper(II) complex by taking 2 equivalents nickel(II) chloride with 1 equivalent ligand. Then 2 equivalents of KPF_6 were added as counter ion. A brown precipitate filtered and dried with diethyl ether (**Scheme-II**). FTIR (ATR, ν_{max}, cm^{-1}): 2865 (Ar C-H), 1768 (C=N),1585 (C=C), 1318 (C-N), 760 (Ni-N). ESR spectra: g = 2.16319.

Antioxidant activity: The antioxidant activity was carried out according to the method of Yamaguchi *et al*. [20], the effect of synthesized ligand and its Cu(II) & Ni(II) complexes on DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity was measured with slight modification and vitamin C was used as the reference standard. Briefly, 0.1 Mm solution



Scheme-II: Synthesis of copper and nickel complexes

of DPPH was incubated with 0-100 µM of ligand and its Cu(II) & Ni(II) complexes for 30 min at ambient temperature in dark and the resulting absorbance was measured using UV/Vis spectrophotometer at 517 nm against a blank (BioMate 3S, Thermo Scientific). The percentage of free radical scavenging was calculated using this formula:

$$\text{DPPH inhibition } (\%) = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times 100$$

Direct hemolytic activity by colourimetric method: Effect of ligand and its Cu(II) & Ni(II) complexes on red blood cells was carried out according to the reported method [21] and the activity was determined by using washed human erythrocytes. Briefly, packed human erythrocytes and phosphate buffered saline (PBS) (1:9v/v) were mixed; 1 mL of this suspension was incubated independently with the various concentration of ligand and its Cu(II) & Ni(II) (0-200 µM) for 1 h at 37 ºC. The reaction was stopped by adding 9 mL of ice cold PBS and centrifuged at1000 g for 10 min at 37 ºC. The amount of hemoglobin released in the supernatant was measured at 540 nm.The activity was expressed as a percent of hemolysis against 100% lysis of cells due to the addition of water that served as positive control and phosphate buffered saline served as negative control.

Antibacterial and antifungal assay: The synthesized ligand and its metal complexes are screened for their antibacterial activity by using agar well diffusion method [22] against pathogenic bacterial strains *Staphylococcus aureus* (NCIM-5022), *Escherichia coli* (NCIM-5051) and antifungal activity by using *Candida albicans* (ATCC-10231) and *Aspergillus niger* (ATCC-1015). Antibacterial studies were conducted by using agar well diffusion method which is based on the diffusion of tested compounds from a well through agar layer in a petri dish. One day before testing, the stock cultures were inoculated in agar or broth media respectively for bacterial and fungal and grown at 37 and 27 ºC for 24 h. Six cups of each 6 mm diameter wells were made into each petri dish with the help of a sterile cork borer and with the help of micropipettes, different concentrations of the standard and the synthesized compound solutions were added into the cups. At 37 ºC, all the plates were then incubated for 24 h. The zone of inhibition of tested compounds of each well was measured in mm was accurately measured and recorded. In order to determine the MIC of compounds, standard drugs and test compounds were diluted to to give a concentration of 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 µg/mL from a stock solution (800 µg/mL). All the samples were inoculated by adding 0.1 mL suspension of bacteria in saline and incubated at the required temperature. MIC was determined by the lowest concentration of sample.

Molecular docking: Ligand and its metal complex molecules were designed and synthesized. The structures were drawn in Chemdraw 11.0 (saved as mol files) and by using ADS, the energies were minimized. The minimized compounds and proteins were saved in structure data(.sd) and protein data bank (PDB) format, respectively for further studies [23]. The docking study was performed using Accelrys Discovery Studio client version 3.5 software (Accelyrs Inc.,http://www.accelrys.com). The X-ray crystallographic structures of all protein (PDB ID 2XCT bound with ciprofloxacin was acquired from the protein data bank (PDB). A grid-based molecular docking method, C-DOCKER algorithm was used to dock the small molecules (ligand and complexes) into the protein active site. The designed structures were submitted to CHARMm (Chemistry at HARvard Macromolecular Mechanics) force field for structure refinement. All water molecules, bound inhibitor and other heteroatoms were removed from the macromolecule and polar hydrogen atoms were added. Energy minimization was carried out for all compounds using CHARMm force field to make stable conformation of protein with an energy gradient of 0.01 kcal/mol/Å. A final minimization of the compounds in the rigid receptor using non-softened potential was performed. For each final pose, the CHARMm energy(interaction energy plus ligand strain) and the interaction energy alone were calculated. The poses were sorted by CHARMm energy and the top scoring (most negative, thus favourable to binding) poses.

RESULTS AND DISCUSSION

The ligand 2,6-*bis*(1,3-thiazol-2-yl)-4-(3,4,5-trimethoxyphenyl)pyridine was synthesized in good yield by slightly modified Kröhnke pyridine synthesis. In the ^{1}H NMR spectrum of ligand, methoxy protons appeared at δ 3.902-3.951 ppm as singlets. Four doublets observed at δ 7.943-7.951 ppm (d, 2H, J = 3.2 Hz), 7.482-7.490 (d, 2H, J =2.8 Hz) are due to protons present in thiazole ring. A singlet observed δ 8.366 ppm due to proton present in pyridine ring. The aromatic protons observed at δ 6.980 ppm (s, 2H, ArH) (Fig. 1). In ^{13}C NMR spectrum, methoxy carbons observed at δ 56.443-60.976 ppm. Aromatic carbons appeared at 104.543, 117.697, 121.978, 133.089 ppm, where as pyridine ring carbon deshielded which observed at 144.06-153.792 ppm. The carbon of thiazole ring highly deshielded which appeared at δ 168.814 ppm (Fig. 2). Mass spectrum M+1 peak observed at 412 (m.w. 411) (Fig. 3). In the FT-IR spectra of complexes, bands observed at 3018 cm^{-1} (arom. -C-H), 1620 (C-O), 1534 (C=C), 1475 (C-N), 2865 (Ar C-H), 1768 (C=N), 1585 (C=C), 1318 (C-N), 552 (Cu-N), 788 (Cu-Cl) shows the formation of copper complex (Fig. 4). Similarly, a Ni-N band appeared at 560 cm^{-1} and a strong peak at 760 cm^{-1} corresponds to P-F bond due to counter ion KPF_6 represents formation of nickel complex (Fig. 5). In the ESR spectrum, g = 2.10753 for CuL_9 (Fig. 6) and g = 2.16319 for NiL_9 (Fig. 7) indicates the presence of free electron and the

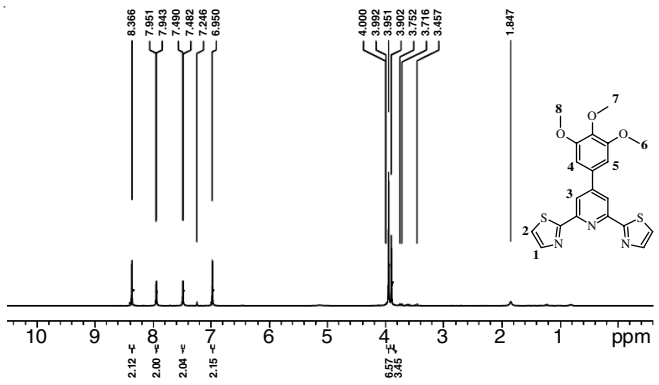


Fig. 1. ^{1}H NMR spectrum of 2,6-*bis*(1,3-thiazol-2-yl)-4-(3,4,5-trimethoxyphenyl)pyridine (L_9) in $CDCl_3$

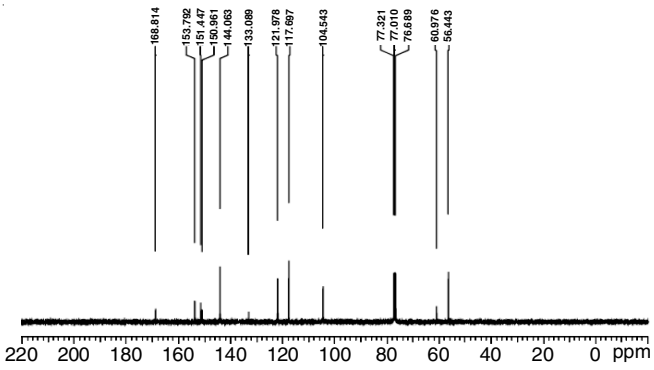


Fig. 2. ^{13}C NMR spectrum of 2,6-*bis*(1,3-thiazol-2-yl)-4-(3,4,5-trimethoxyphenyl)pyridine (L_9) in $CDCl_3$

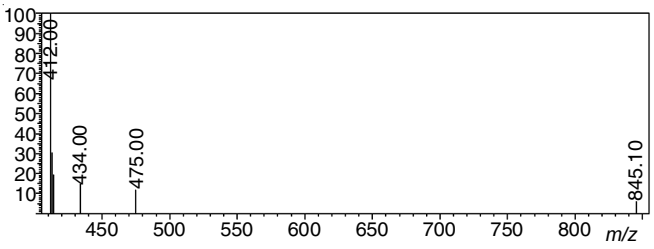


Fig. 3. Mass spectrum 2,6-*bis*(1,3-thiazol-2-yl)-4-(3,4,5-trimethoxyphenyl)pyridine (L_9)

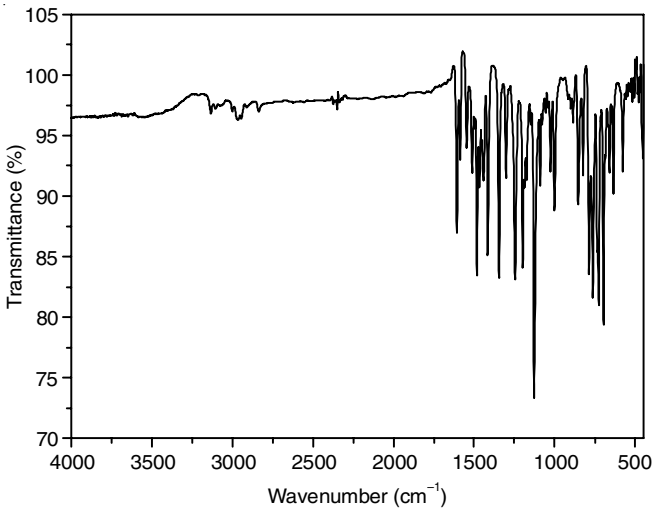


Fig. 4. FT-IR spectrum of copper complex (CuL_9)

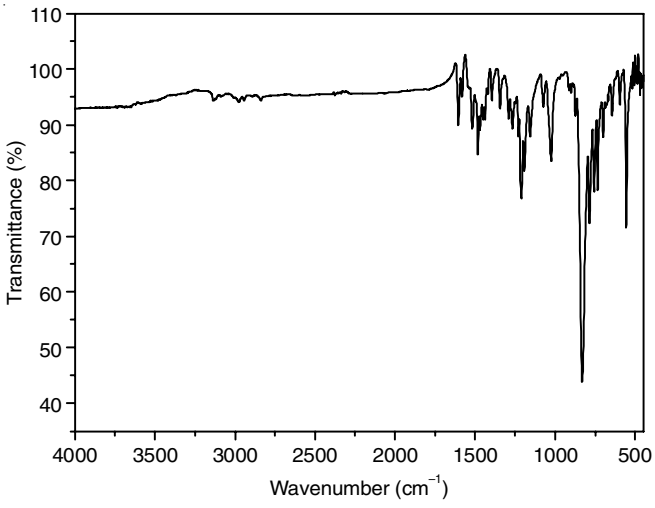


Fig. 5. FT-IR spectrum of nickel complex (NiL_9)

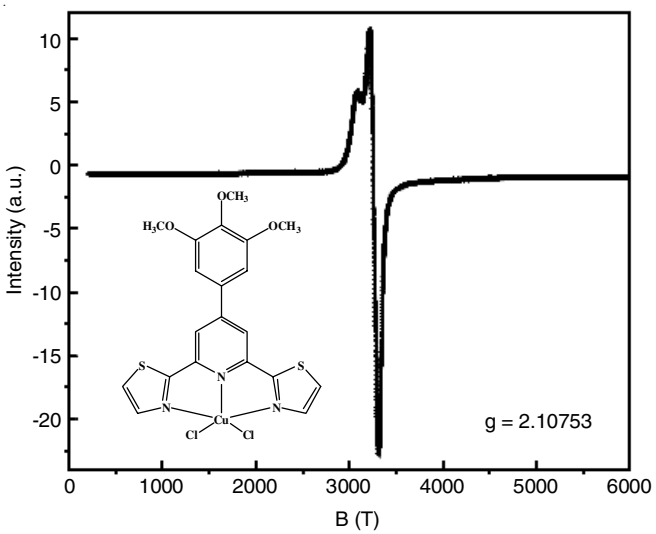


Fig. 6. EPR spectrum of copper complex (CuL_9)

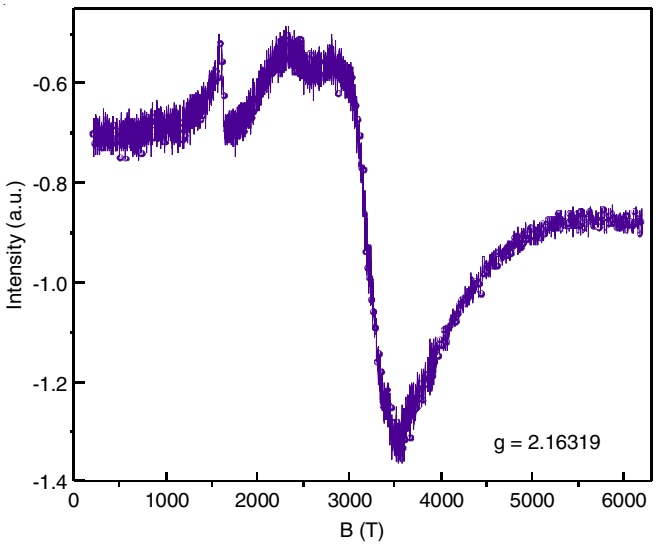


Fig. 7. ESR spectrum of nickel complex (NiL_9)

complex is paramagnetic with distorted square planar geometry for CuL_9 and distorted octahedral geometry for NiL_9.

Antibacterial and antifungal assay: All the synthesized compounds showed a significant antimicrobial activity against tested bacterial strains and fungal strains. Interestingly, copper complexes showed more antimicrobial activity as compared with the ligand, while nickel complex showed a mild antimicrobial activities (Table-1).

TABLE-1
ANTIMICROBIAL ACTIVITIES OF SYNTHESIZED COMPOUNDS

Compound	Bacterial strains		Fungal strains	
	S. aureus	*E. coli*	*C. albicans*	*A. niger*
L_9	8.5	10.0	9.00	9.52
CuL_9	10.38	11.05	9.35	11.35
NiL_9	9.80	10.04	9.32	9.34
Ciprofloxacin	24.0	24.0	–	–
Fluconazole	–	–	25.0	25.0

Antioxidant activity: The synthesized ligand and its Cu(II) & Ni(II) complexes were evaluated for their free radical scaven-

ging potentiality. Compounds showed good free radical scavenging activity when compared with control vitamin C. Based on these results, the ligand and its Cu(II) & Ni(II) complexes were considered as a potent candidates for stress reduction (Table-2, Fig. 8).

TABLE-2
DPPH SCAVENGING OF L_9, CuL_9 AND NiL_9

Compound/ Concentration	Scavenging (%)					
	L_9		CuL_9		NiL_9	
DPPH	93.00	88.00	95.00	90.00	92.00	88.00
20 µM	85.00	81.00	85.00	80.00	90.00	85.00
40 µM	75.00	70.00	73.00	78.00	81.00	76.00
60 µM	63.00	57.00	60.00	55.00	65.00	60.00
80 µM	43.00	38.00	46.00	41.00	40.00	35.00
100 µM	35.00	30.00	33.00	28.00	33.00	37.00
Vitamin C	10.00	15.00	15.00	10.00	10.00	15.00

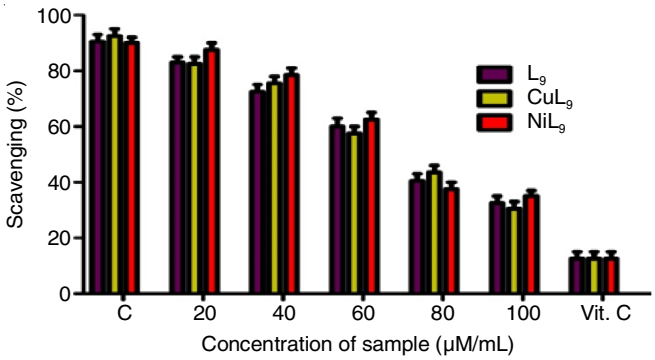


Fig. 8. Graphical representation of DPPH scavenging of L_9, CuL_9 and NiL_9

Hemolytic activity: Effect of synthesized ligand and its Cu(II) & Ni(II) complexes on red blood cells was carried out by using washed human erythrocytes. The compounds show the

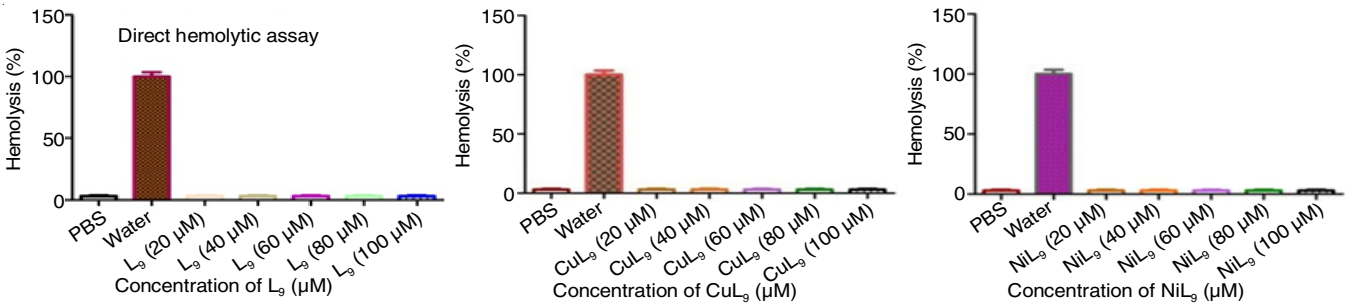


Fig. 9. Hemolytic activity of L_9, CuL_9 and NiL_9

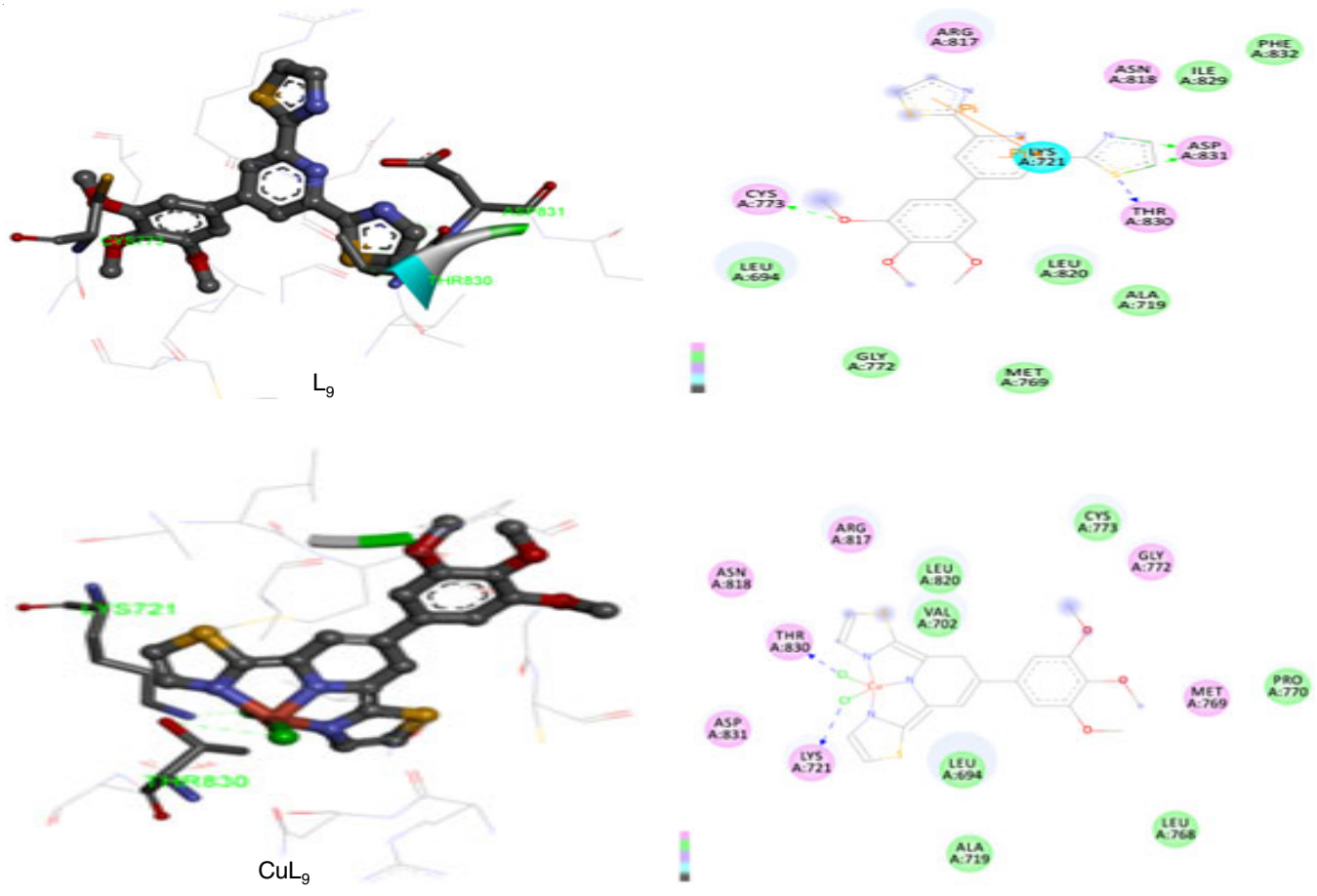


Fig. 10. Binding pattern of L_9, CuL_9 with target protein 2XCT

non-toxic to RBC as it did not hydrolyze the RBC membrane when compared with the positive control water (Fig. 9).

Molecular docking study: Synthesized ligand (L_9) and the copper complex showed a good binding energy (Fig. 10) whereas nickel complex did not show any effective binding. The binding energy value of the synthesized ligand and its Cu(II) & Ni(II) complexes are shown in Table-3.

TABLE-3
BINDING ENERGY OF THE COMPOUNDS

Compound	C Docker Energy
L_9	-90
CuL_9	-84.8
NiL_9	0

Conclusion

A novel ligand 2,6-*bis*(1,3-thiazol-2-yl)-4-(3,4,5-trimethoxyphenyl)pyridine and its copper(II) and nickel(II) complexes were synthesized in good yield. All the compounds were characterized by spectroscopic and analytical methods. The synthesized ligand and metal complexes found to be biologically potent molecules as they possess significant antibacterial, antifungal, antioxidant activities and docking energies. Moreover, synthesized ligand and its Cu(II) & Ni(II) exhibit non-toxicity as they did not cause any effect to human erythrocyte.

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