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Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

SYNTHESIS, CHARACTERIZATION, BIOLOGICAL ACTIVITIES OF NOVEL TRIDENTATE LIGAND AND ITS CU(II) & NI(II) COMPLEXES

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ABSTRACT

In the present study, we reported a novel (NNN) type ligand 2,6-bis(1,3-thiazol-2-yl)-4-(2,4,5-trimethoxyphenyl)pyridine and its Cu(II) and Ni(II) complexes. The synthesized were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, Mass, UV and ESR spectra. The antioxidant activity of the synthesized compounds were evaluated by the percentage of inhibition of 1,1-diphenyl-2-picryl hydrazyl (DPPH) and compounds found to be potent antioxidants. The synthesized compounds screened for their antimicrobial activity and results showed that the synthesized compounds showed mild antimicrobial activity in comparison with standard drugs. Copper complexes showed good antimicrobial activity than ligand as well as Nickel complex. Fascinately, ligand and its complexes exhibit non-toxic property as they did not cause any effect to human erythrocyte, which shows its nontoxic nature.

Keywords: Ligand, Antioxidant, Hemolysis, Antimicrobial Activity, Molecular Docking

1. INTRODUCTION

Thiazoles and its derivatives possess spectrum of biological and pharmaceutical activities, such as anti-inflammatory, antimicrobial, antioxidant, antifungal, anticancer, antiviral, antitumor, antidiabetic , anticonvulsant, antitubercular [1-3] etc. The discovery of tridentate ligand 2,2':6',2''-terpyridines (tpy) which attracted the attention of researchers due to its excellent coordinating capacity with various transition metals and lanthanides. Compounds containing acetylpyridine, acetylpyrazine or acetylthiazole are the starting material for the formation of a tridentate ligand by Kroenke pyridine synthesis. [4-6]. Many novel ligands and metal complexes were reported on 2,2':6',2''-terpyridines derivatives but only few ligands and complexes of 4-(aryl)-modified-2,6-di(1,3-thiazol-2-yl) pyridine were known [7-8]. These ligands and their various transition metal complexes were studied for their various biological, photophysical and pharmacological activities like anticancer activity, DNA interaction, DNA binding, DNA cleaving agents, cytotoxicity, DFT calculations, photoluminescence and catalytic activity, antitumor, antimicrobial, or anti-HIV agents [9-11].

Also, metal complexes of 4-(aryl)-modified-2,6-di(1,3-thiazol-2-yl) pyridine have the capability to form complexes with various transition metals and in view of their interesting photophysical, magnetic, photonic, electronic and structural properties, as well as challenging applications in catalysis, supramolecular chemistry, molecular magnetism, molecular electronics etc. Especially copper complexes have attracted significant attention due to spectrum of biological activities [12]. So, in continuation of our work on novel tridentate ligands and their transition metal complexes, in the present study, we reported novel ligand and its Cu and Ni complexes. Due to stress conditions in our life free radical molecules are generated which are main damaging particles. These free radicals mainly lead to the progression of many pathological events. Damaging-free radical generation is the key event in the progression of many compulsive conditions. Throughout the condition of oxidative stress, these generated free radicals contribute to the major role in inducing cell mediated death. So research focused to find novel antioxidants. Similarly search for potential antimicrobial agents always been a key research interest.

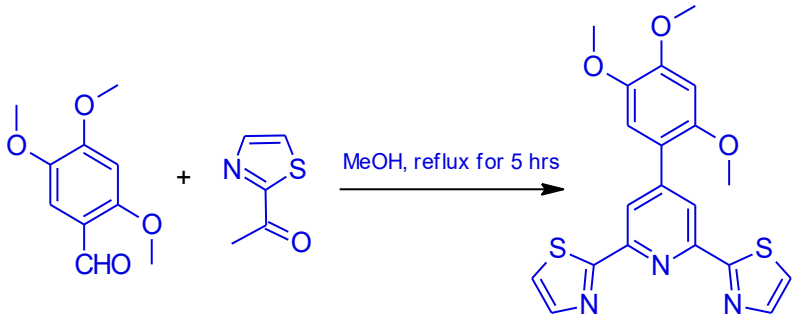
2. EXPERIMENTAL

2.1. Material and Methods

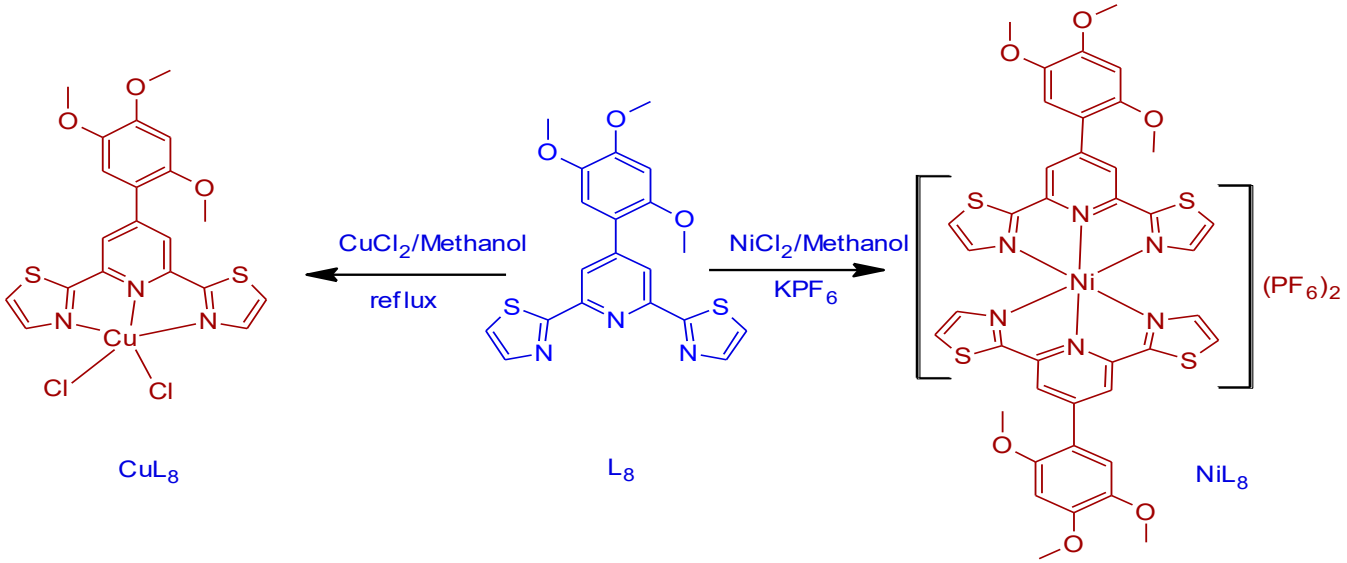
All the reagents required for the synthesis were purchased commercially from Merck and Sigma Aldrich and used without any further purification. Solvents obtained from Spectrochem and were of analytical grades. Melting points of the compounds were recorded on a hot stage Gallen Kamp melting point apparatus.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

2.2. Preparation of 2,6-bis(1,3-thiazol-2-yl)-4-(2,4,5-trimethoxyphenyl)pyridine (L_8)

In a 100 mL two neck round-bottom flask, 2 mmol of 2-Acetylthiazole dissolved in Methanol (30 mL). To this, KOH pellets (0.560 g, 4 mmol) and 2 mL of water added. The reaction mixture was stirred for 30 minutes and then added 2,4,5-trimethoxybenzaldehyde (1 mmol) dissolved in methanol added and continued stirring for 5 hrs as per the Khronke pyridine synthesis [13]. The solid was filtered and washed with methanol and then diethyl ether. The yellow colored solid with 75% yield obtained. The ligand obtained used for complexation without further purification.



Scheme1: Synthesis of 2,6-bis(1,3-thiazol-2-yl)-4-(2,4,5-trimethoxyphenyl)pyridine (L_8)



Scheme 2: Synthesis of Copper(CuL_8) and Nickel(NiL_8) complexes

2.3. Preparation of Copper metal complexes (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 the L_8. The mixture was refluxed at 60°C temperature 4 hours. The green precipitate was collected by filtration [14]. The collected precipitate dried with diethyl ether, recrystallized in methanol chloroform (1:1) mixture.

2.4. Preparation of Nickel metal complex (M:L=1:2)

Nickel complexes were prepared by taking two equivalents of Nickel Chloride with one equivalent of ligand by the same procedure as Copper complex. Finally two equivalents of KPF_6 were added as counter ion [14]. A brown precipitate filtered and dried with diethyl ether.

2.5. Antioxidant activity

The antioxidant activity was carried out according to the method of Yamaguchi et al. [15], the effect of L_8, CuL_8, and NiL_8 on DPPH(2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity was measured with slight change in the methodology and vitamin C was used as the reference standard. Briefly, 0.1 Mm solution of DPPH was incubated with 0–100µM of L_8, CuL_8, and NiL_8 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.

% DPPH inhibition = [(OD of control –OD of test) /(OD of control)]x100

2.6. Direct hemolytic activity by the colourimetric method

Effect of L_8, CuL_8, and NiL_8 on red blood cells was carried out according to the method Jayanna kengaiah et al. [16] the activity was determined by using washed human erythrocytes. Briefly, packed human erythrocytes and phosphate buffered saline (PBS) (1:9v/v) were mixed; 1ml of this suspension was incubated independently with the various concentration of L_8, CuL_8, and NiL_8 (0-200µM) for 1h at 37°C. The reaction was stopped by adding 9ml of ice cold PBS and centrifuged at1000g for 10min at 37°C. 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 which served as positive control and phosphate buffered saline served as negative control.

2.7. Antibacterial and antifungal assay

Thiazole and its derivatives are found to exhibit various biological and pharmaceutical activities, such as antifungal, antibacterial and hence all the synthesized compounds were screened for their antimicrobial evaluation. The synthesized ligand and metal complexes were screened for their antibacterial activity by using agar well diffusion method [17] against pathogenic bacterial strains *Staphylococcus aureus* (NCIM-5022), *Escherichia coli* (NCIM-5051), and antifungal activity was done 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°C and 27°C for 24 hours. 6 cups of each 6 mm diameter wells were made into each Petri dish with the help of a sterile cork borer .With the help of micropipettes, different concentrations of the standard and the synthesized compound solutions were added into the cups. At 37°C temperature, all the plates were then incubated for 24h. 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 (800µg/ml). All the samples were inoculated by adding 0.1ml suspension of bacteria in saline and incubated at the required temperature. MIC was determined by the lowest concentration of sample. Compounds showed significant antimicrobial activity against different strains tested .The results obtained for antibacterial, antifungal activities were given in Table 1.

2.8. Molecular Docking study

Ligand and complexes 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 [18]. 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 HARvardMacromolecular

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 favorable to binding) poses. The binding energy of the compounds was as given in Table 3. Binding interaction was as given in figure 10. Compounds show good binding energy.

3. RESULTS AND DISCUSSION

The ligand 2,6-bis(1,3-thiazol-2-yl)-4-(2,4,5-trimethoxyphenyl)pyridine was prepared in good yield by slightly modified Kronke Pyridine synthesis.

3.1.Spectral analyses

In the **^{1}H NMR** spectrum (Fig. 1) of ligand, methoxy protons appeared at δ 3.832, δ 3.896, δ 3.950 as singlets. Four doublets observed at 7.943-7.951 (d, 2H, J = 3.2 Hz), 7.455-7.463 (d, 2H, *J* =2.8 Hz) are due to protons present in thiazole ring. A singlet observed δ 8.371 (s, 2H) due to proton present in pyridine ring. The aromatic protons observed at δ 7.027 (s, 1H, ArH), 6.620 (s, 1H, ArH).

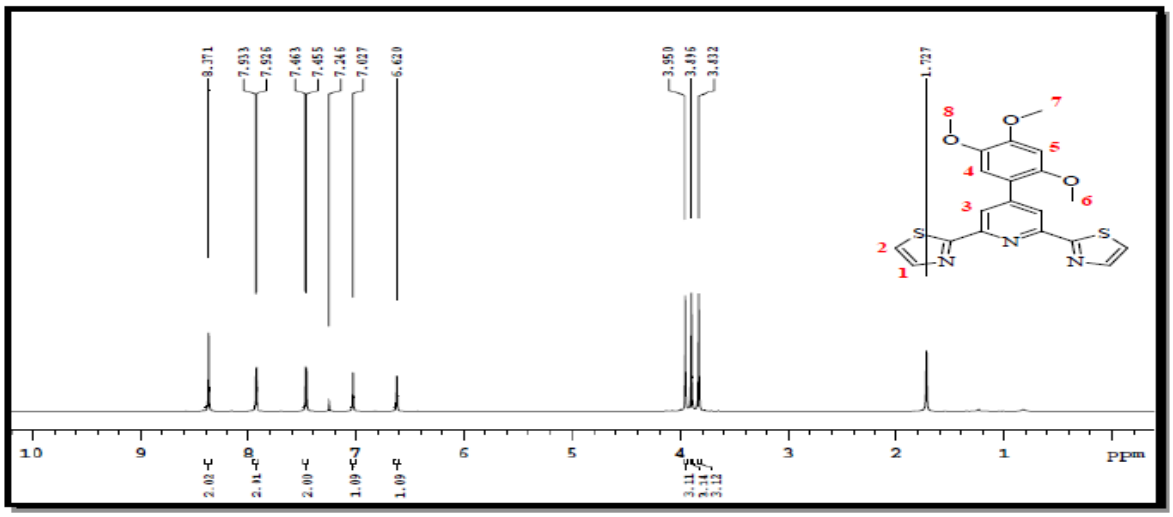


Fig. 1: ^{1}H NMR spectrum of 2,6-bis(1,3-thiazol-2-yl)-4-(2,4,5-trimethoxyphenyl)pyridine (L_8) in $CDCl_3$

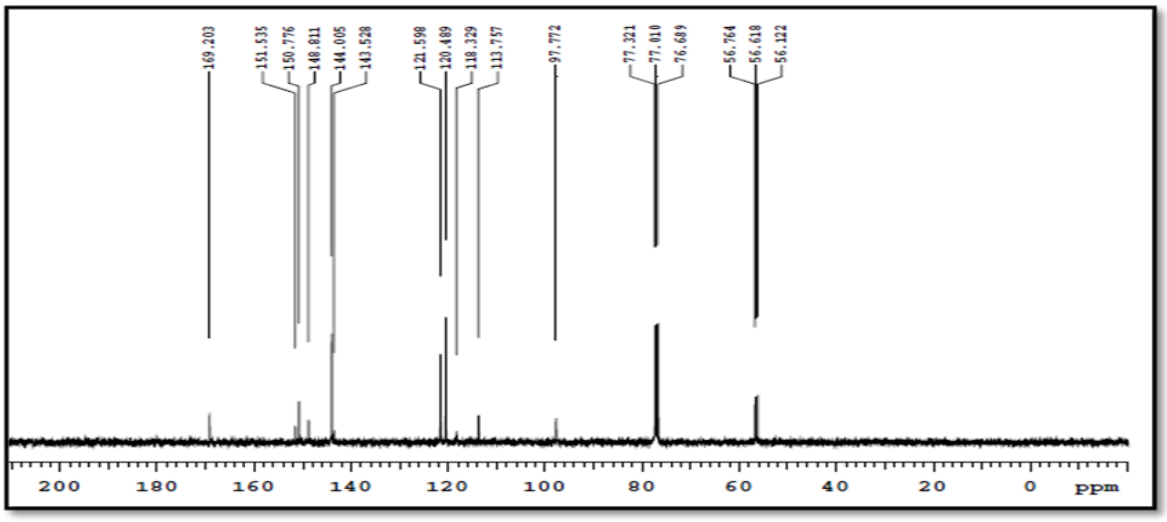


Fig. 2: ^{13}C NMR spectrum of 2,6-bis(1,3-thiazol-2-yl)-4-(2,4,5-trimethoxyphenyl)pyridine (L_8) in $CDCl_3$

In the **^{13}C NMR** (Fig. 2) methoxy (OCH_3) carbons observed at 56.122-56.764 ppm. Aromatic carbons appeared 97.772, 113.757, 118.329, 120.489, 121.598 ppm where as pyridine ring carbon deshielded which observed at 143.528-151.535 ppm. The carbon of thiazole rind highly deshielded which appeared at 169.203 ppm.

The **Mass Spectrum** (Fig. 3) M+1 peak observed at 412 (Molecular weight 411).

In the **FT-IR** spectrum (Fig. 4 & 5) of CuL_8, NiL_8 a weak peak observed at 3020 cm^{-1} for aromatic –C-H, 1621 cm^{-1} due to (C-O), 1534 cm^{-1} corresponds to (C=C), 1475 cm^{-1} due to (C-N), 1768 cm^{-1} due to (C=N),1585 cm^{-1} (C=C), 1318 cm^{-1} (C-N), 760 The Cu-N band is observed in the region of 552 and Cu-Cl at 793 cm^{-1} shows the formation of copper complex. The Ni-N bond appeared at 572 cm^{-1} and a strong peak at 790 cm^{-1} corresponds to P-F bond due to counter ion KPF_6 represents formation of nickel complex.

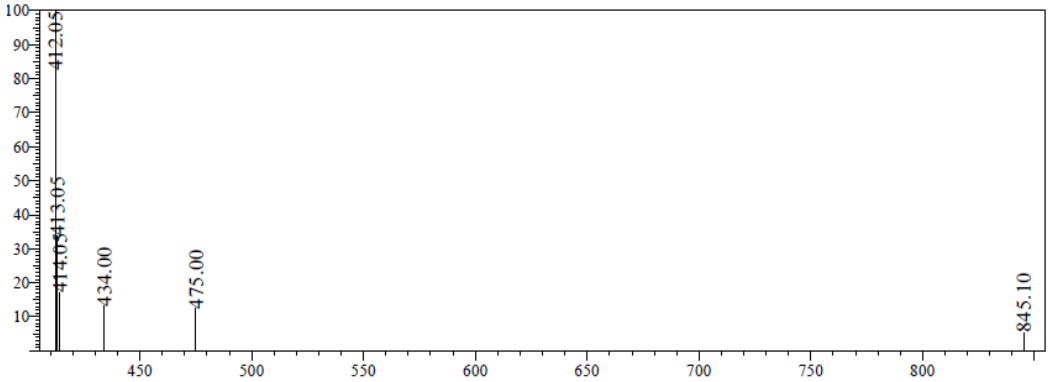


Fig. 3: Mass Spectru6 2,6-bis(1,3-thiazol-2-yl)-4-(2,4,5-trimethoxyphenyl)pyridine (L_8)

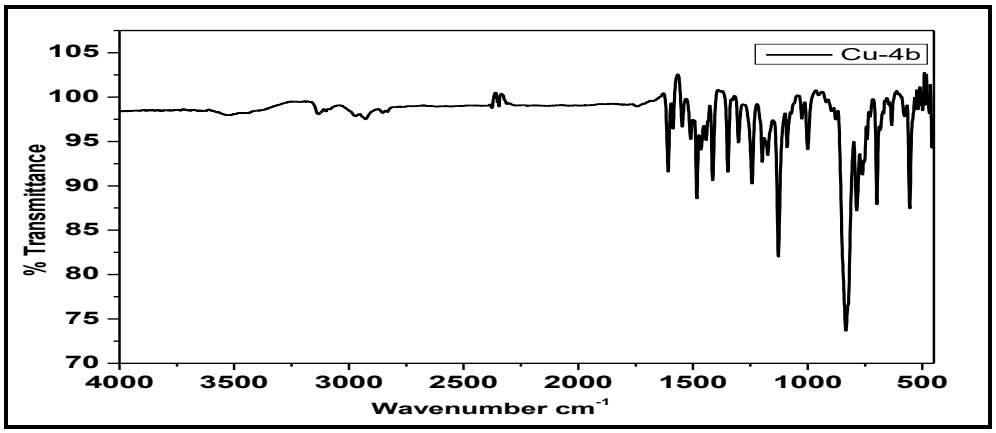


Figure 4: FT-IR Spectrum CuL_8

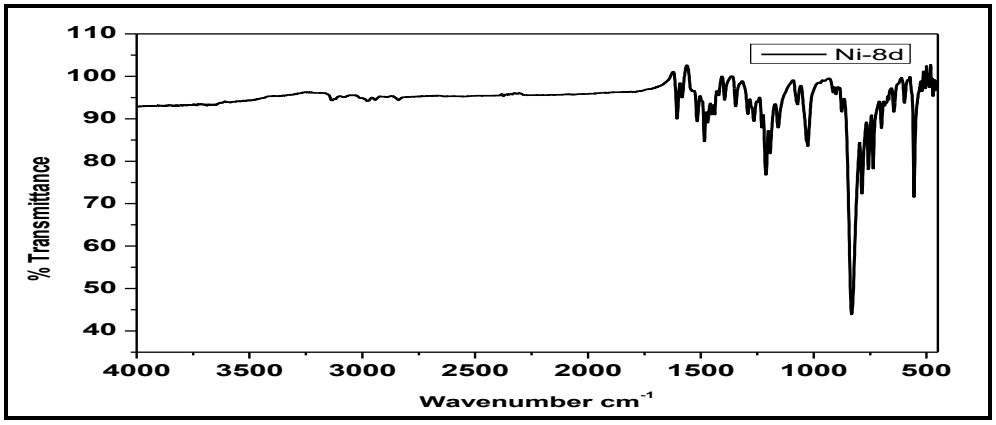


Fig. 5: FT-IR Spectrum NiL_8

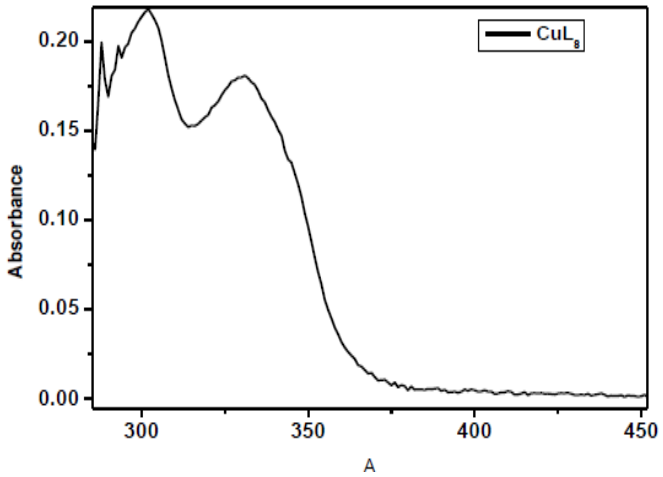


Fig. 6: UV-Visible Spectrum of CuL_8,

UV-Vis.: λ_{max}: Absorption bands at 295, 350 nm attributed due to d-d transitions, π-π* and n-π* transitions in copper complexes (Fig. 6).

In the ESR Spectrum (Fig. 7) of NiL_9, the value of g=2.15354 indicates the presence of free electrons and hence the complex is paramagnetic with distorted octahedral geometry.

The synthesized ligand and complexes were well characterized by ^{1}H NMR, ^{13}C NMR, Mass, FT-IR, UV and ESR spectra. The experimental data obtained was in good correlation with the theoretical values. Hence the assigned structures were confirmed

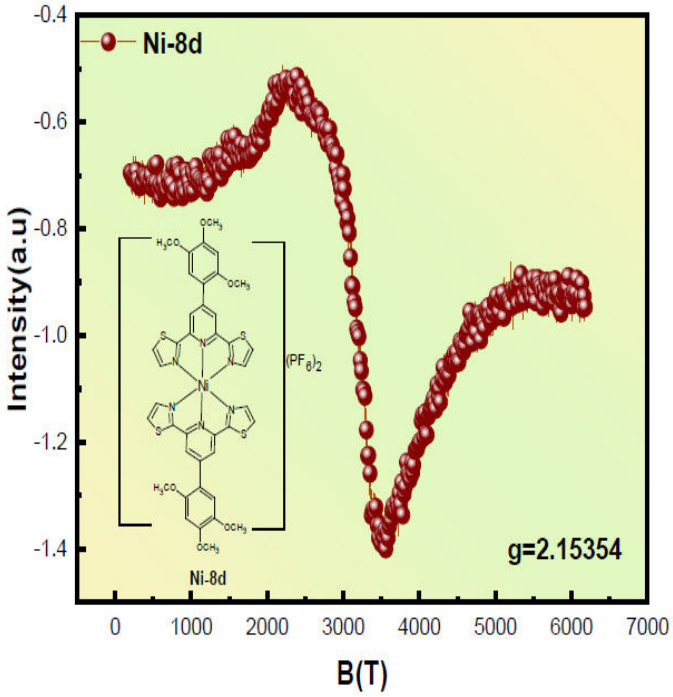


Fig. 7: ESR Spectra of NiL_8

3.2. Antibacterial and antifungal assay

All the prepared compounds showed moderate antimicrobial activity against tested bacterial strains and fungal strains (Table 1). Interestingly, it is observed that Copper complexes showed good antimicrobial activity in comparison with the ligand and Nickel complexes.

Table 1 : Antimicrobial activities of synthesized compounds

Compound	Bacterial strains		Fungal strains	
	S. aureus	*E. coli*	*C. albicans*	*A. niger*
L_8	10.22	10.81	7.21	8.32
CuL_8	12.48	13.86	10.36	10.47
NiL_8	9.24	9.45	8.21	8.76
Ciprofloxacin	24.0	24.0	-	-
Fluconazole	-	-	25.0	25.0

Table 2: DPPH Scavenging of L_8, CuL_8 and NiL_8

Compound/Concentration	% Scavenging					
	L_8		CuL_8		NiL_8	
DPPH	85.00	90.00	85.00	90.00	85.00	90.00
20 µM	82.00	86.00	78.00	74.00	80.00	76.00
40 µM	77.00	72.00	70.00	65.00	75.00	70.00
60 µM	63.00	57.00	60.00	55.00	65.00	60.00
80 µM	46.00	41.00	43.00	38.00	49.00	44.00
100 µM	35.00	30.00	36.00	31.00	35.00	30.00
Vitamin C	13.00	10.00	15.00	12.00	12.00	9.00

3.3. Antioxidant activity

The synthesized ligand and complexes were evaluated for their free radical scavenging activities. Compounds showed good free radical scavenging activity when compared with known positive control vitamin C. The results obtained shows that the compounds L_8, CuL_8 and NiL_8 were considered as potential antioxidant agents (Table 2, Fig. 8).

3.4. Hemolytic activity

Effect of synthesized compounds on red blood cells was carried out by using washed human erythrocytes. The compounds did not showed any damage to RBC as it did not hydrolyze the RBC membrane that was compared with the positive control water (Fig. 9).

3.5. Molecular Docking study

Synthesized ligand and copper complex showed good binding energy whereas nickel complexes did not show any binding energy.

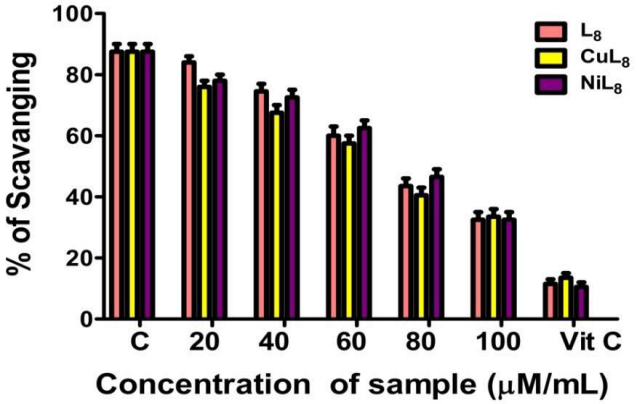


Fig. 8: Graphical representation of DPPH Scavenging of L_8, CuL_8 and NiL_8

Table 3: Binding energy of the compounds

Compound	C Docker Energy
L_8	-104
CuL_8	-90.3
NiL_8	0

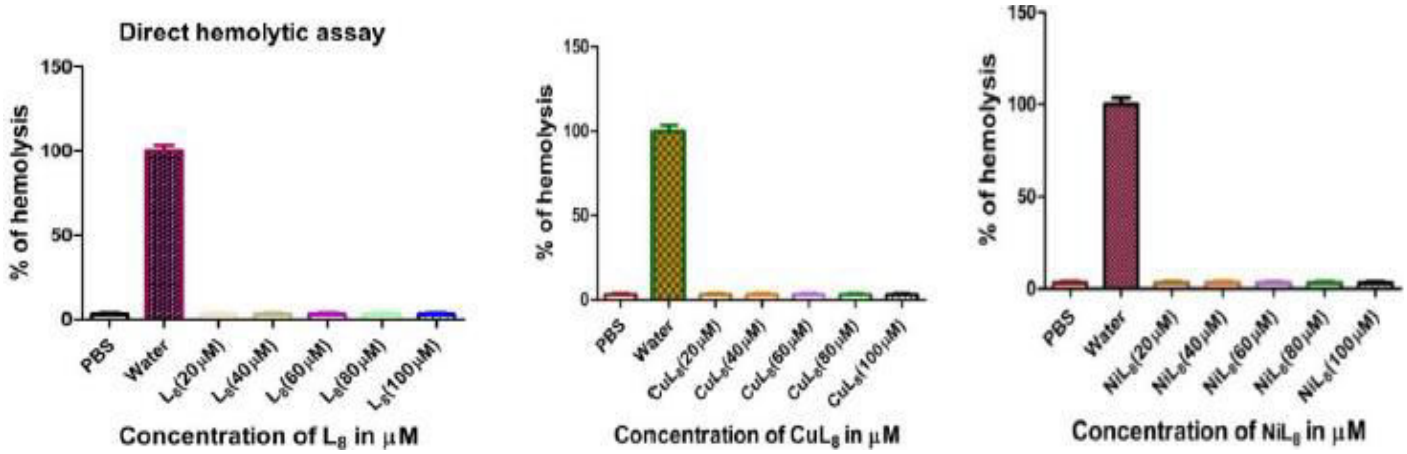
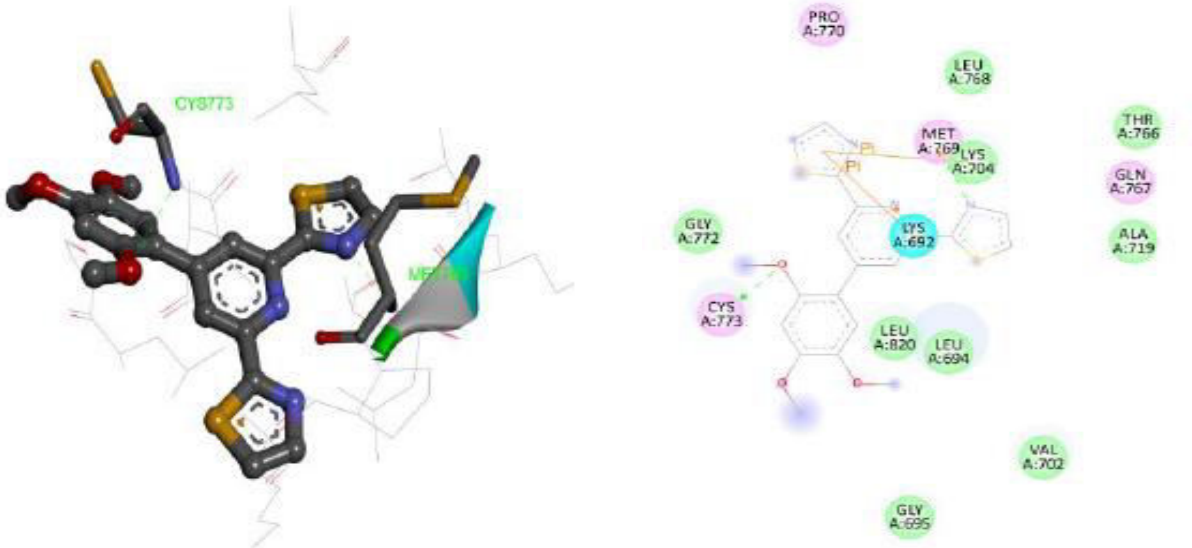


Fig. 9: Hemolytic activity of L_8, CuL_8 and NiL_8



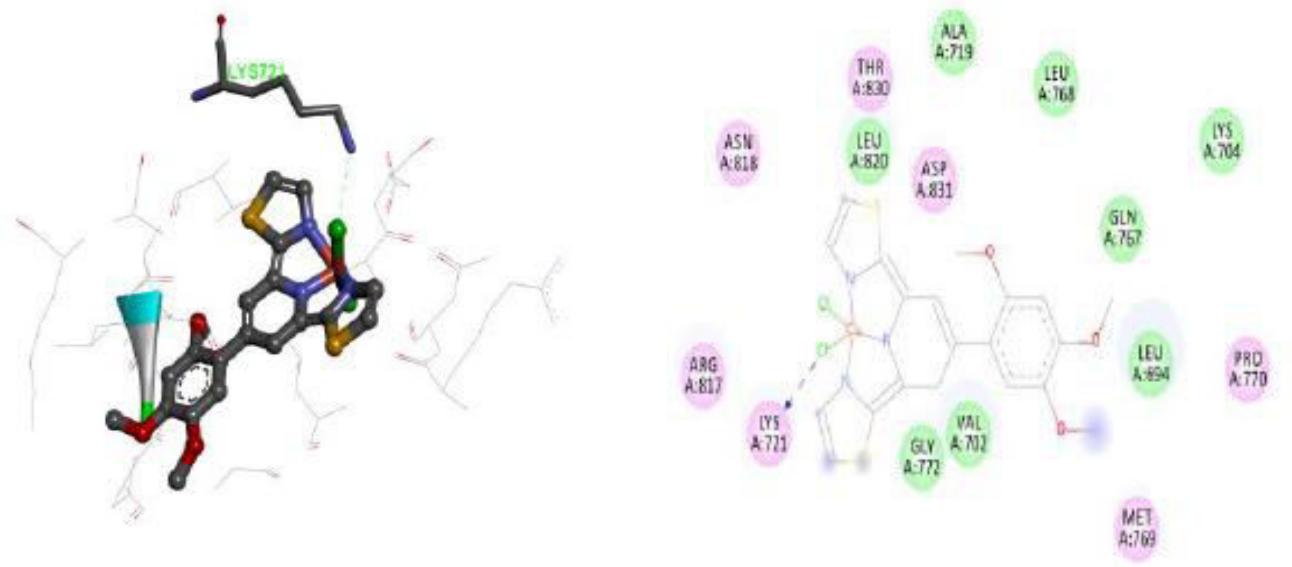


Fig. 10: Binding pattern of L_8 and CuL_8 with target protein 2XCT

4. CONCLUSIONS

The novel ligand 2,6-bis(1,3-thiazol-2-yl)-4-(2,4,5-trimethoxyphenyl)pyridine and the Copper and Nickel complexes were synthesized in good yield and well characterized by spectroscopic and analytical methods. The synthesized compounds $L_{8,}$ CuL_8 and NiL_8 found to be biologically potent molecules as they possess various activities such as antibacterial, antifungal, antioxidant activities and docking energies. Finally the compounds showed nontoxicity.

5. ACKNOWLEDGEMENTS

The authors are thankful to Dr Raghavendra Kumar for providing lab facility. Also, the authors are grateful Dr Devaraja, Department of Biochemistry for providing lab facility and support to study bacterial, fungal, and antioxidant activities.

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