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Research Article

STRUCTURAL CHARACTERIZATION, ANTIMICROBIAL, ANTIOXIDANT, MOLECULAR DOCKING STUDIES OF NOVEL 4-(2,4-DIMETHOXYPHENYL)-2,6-BIS(1,3-THIAZOL-2-YL)PYRIDINE AND ITS CU(II) &NI(II) COMPLEXES

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ABSTRACT

In the present study, we reported a novel ligand 4-(2,4-dimethoxyphenyl)-2,6-bis(1,3-thiazol-2-yl)pyridine and its Cu(II) and Ni(II) complexes were prepared. All the synthesized compounds have been characterized by ^{1}H NMR, ^{13}C NMR, Mass, UV, FT-IR, and ESR spectra. Synthesized ligand and its Cu(II) and Ni(II) metal complexes were screened for their antimicrobial and the compounds showed mild activity in comparison with a standard. Also, all the synthesized compounds were studied for their antioxidant activity and hemolytic activity. The antioxidant activity of the ligand (SM-2b) and its metal complexes were evaluated by the reduction of 1,1-diphenyl-2-picryl hydrazyl (DPPH) and compounds found to be potent antioxidants. Interestingly, ligand and its complexes exhibit non-toxic property as it did not cause any effect to human erythrocyte, suggesting its nontoxic nature.

Keywords: Ligands, Complexes, antioxidant activity, hemolysis, antimicrobial activity.

1. INTRODUCTION

The thiazole nucleus is a very potent heterocycle in many biologically active compounds. It is one of the extensively studied heterocycles [1]. Thiazole and its derivatives play vital roles in many drugs. Thiazoles and its derivatives possess various biological and pharmaceutical activities, such as antifungal, antibacterial, anti-inflammatory, antiviral, antioxidant, antitumor, antidiabetic and antitubercular, etc. [2-9]. Since the discovery of ligand 2,2': 6',2''-terpyridines (tpy), have attracted widespread attention of Chemists due to their excellent coordinating/complexing capacity as N-donor ligands towards various transition-metal and lanthanide cations [10-14]. Compounds containing acetylpyridine or acetylthiazole are the starting material for the formation of a ligand by Kroenke pyridine ligand synthesis. [15-19]. Many 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 [20-24]. To date, only a few 2,6-di(thiazol-2-yl)pyridine derivatives and their transition-metal complexes have been reported [25-27].

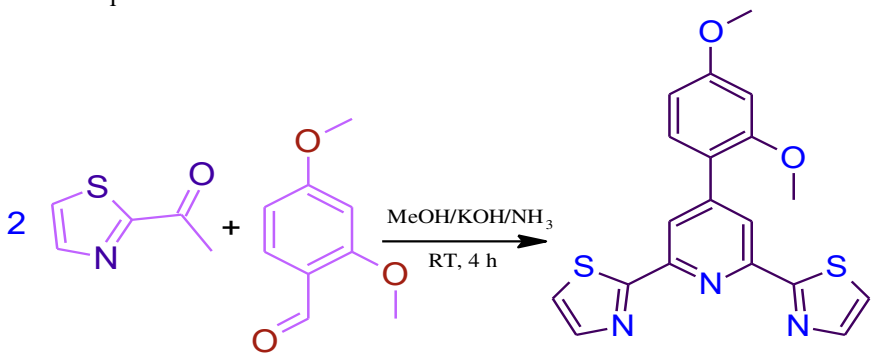
Metal complexes of 4-(aryl)-modified-2,6-di(1,3-thiazol-2-yl) pyridine have also attracted widespread scientific attention because of their capability to form complexes with various transition metals and in view of their interesting photophysical, electronic, photonic, magnetic, reactive and structural properties, as well as promising applications in supramolecular chemistry, catalysis, molecular magnetism, molecular electronics, and anti-tumor therapy. These ligands and their various transition metal complexes have been extensively studied for their photophysical and various biological & 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 [28-34]. Many copper complexes have attracted significant attention due to the reason that they exhibit various biological activities like DNA binding, DNA cleaving agents, anticancer, antimicrobial, antioxidants., etc. [19, 24-25, 31-34]. In the present study, novel ligand and its Cu and Ni complexes have been reported.

2. MATERIAL AND METHOD

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 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 was carried out by a standard method and UV spectra were recorded using UV spectrophotometer. Mass spectrum was recorded on Mass Spectrophotometer. Elemental analysis was done by conventional methods.

2.1. Preparation of 2,2'-(4-(2,3-dimethoxyphenyl) pyridine-2,6 diyl)dithiazole (SM-2b):

2-Acetylthiazole (2 mmol) taken in a 100 mL round-bottom flask, was added with MeOH (30 mL), KOH pellets (0.560 g, 4 mmol) and 2 mL of water. The mixture was stirred for 10 min and then added the corresponding 2,3-dimethoxybenzaldehyde (1 mmol) at room temperature with continued stirring for 4 h [35]. The solid was filtered and washed with methanol followed by diethyl ether. The yellow colored solid with 85% yield was obtained. The ligand obtained was used for complexation without further purification.



SM-2b

Scheme 1: Synthesis of Ligand (SM-2b)

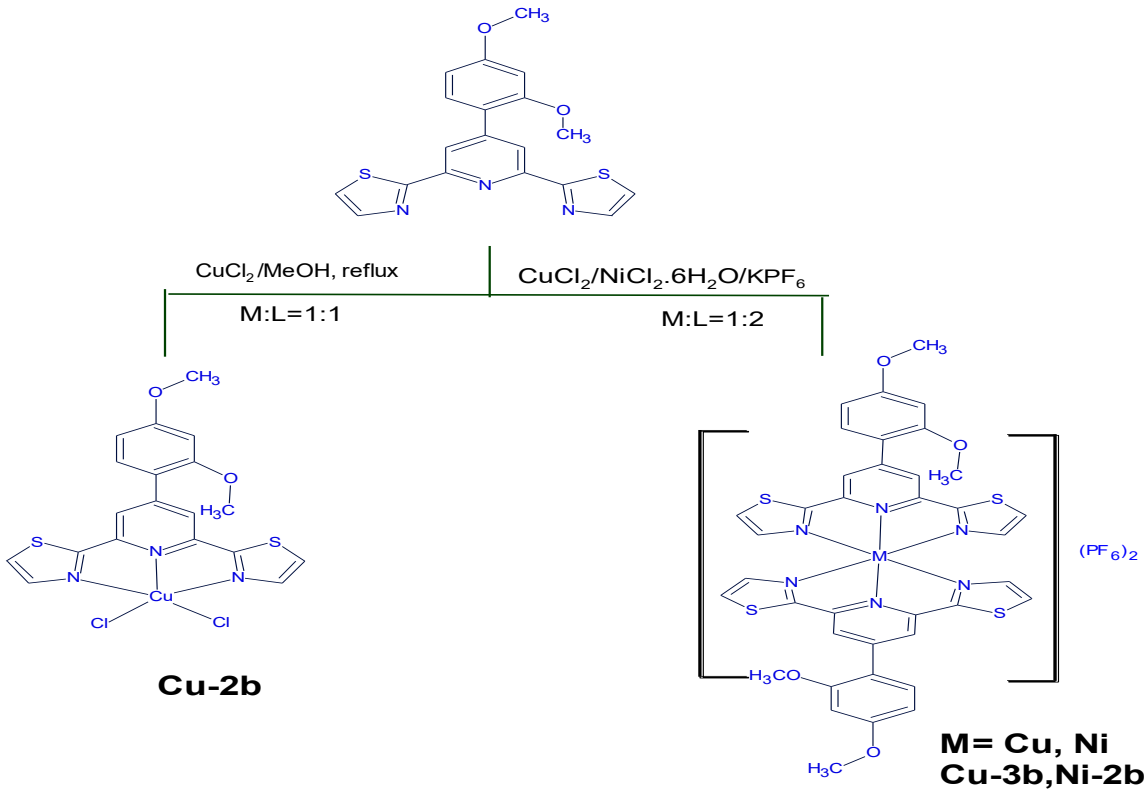
^{1}H NMR (400 MHz, d6-DMSO): δ 8.377 (s, 2H, $H^{B3,5}$), 7.927-7.935 (d, 2H, J = 3.2 Hz, H^{A2}), 7.454-7.461 (d, 2H, J =2.8 Hz, H^{A3}), 7.438 (s, 1H, ArH), 6.596-6.602 (d, 1H, ArH), 6.565-6.575 (d, 1H, ArH), 3.865 (s, 3H, OCH_3), 3.853 (s, 3H, OCH_3); **^{13}CNMR** (100 MHz, δ ppm): 169.271, 161.828, 157.995, 150.727, 148.801, 143.985, 131.347, 121.482, 120.480, 104.991, 55.693, 55.450; **FT-IR** (ν, cm^{-1}): 3057, 2827, 1688, 1586, 1547, 1495, 1456, 1417, 1371, 1326, 1280, 1218, 1127, 1042, 889, 861, 759. **m/z** 381, M^+ 382.

2.2. Preparation of Copper metal complexes (M:L=1:1)

A solution of $CuCl_2 \cdot 2H_2O$ (1 mmol) was dissolved in 10 mL methanol and was added to a hot methanolic solution (10 mL) of the 2,2'-(4-(2,4-dimethoxyphenyl)pyridine-2,6-diyl)dithiazole (SM-2b). The mixture was refluxed at 50°C temperature for 5-6 hrs. The green precipitate was collected by filtration [27]. The collected precipitate was dried with diethyl ether and recrystallized in methanol chloroform (1:1) mixture. The product obtained was identified by **IR spectra**.Cu(sm-2b) Cl_2: 3018, 1620, 1534, 1475, 1420, 1262, 1250, 1142, 1020, 968, 788. **UV-Vis.:** λ_{max}: 312 nm

2.3. Preparation of Copper/Nickel metal complexes (M:L=1:2)

Cu-3b and **Ni-2b** complexes were prepared by the same procedure as described for Cu-2b by taking 2 equivalents Metal Chloride with 1 equivalents ligand. Then 2 equivalents of KPF_6 were added as counter ion. A green precipitate was obtained which was filtered and dried with diethyl ether [33]. Copper complex, **[Cu-2b]:** Dark Green solid: **FT-IR** (ν, cm^{-1}): 3020, 2670, 1650 (C=N), 1605(C=C), 1320(C-N); **UV-Vis.:** λ_{max}: 465 nm, **Nickel complex, [Ni-2a]:** light green solid: **FTIR** (ν,cm^{1}):2815, 1738(C=N),1575(C=C), 1318(C-N); **UV-Vis.:** λ_{max}: 496 nm.



Scheme 2: Synthesis of Metal Complexes Cu-2b, Cu-3b, Ni-2b.

2.4. 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 [6] against pathogenic bacterial strains *Staphylococcus aureus* (MTCC 96), *Escherichia coli* and antifungal activity by using *Aspergillus niger* (MTCC 1344), *Candida albicans* (MTCC 854). For bacterias, the samples were incubated at 36°C for 24 hours. For fungi, the samples were incubated at 25°C for seven days for A. Niger and C. albicans, the samples incubated at 37°C for two days. The diameters of zone of inhibition at MIC of 100µg/mL were measured in mm concerning the ciprofloxacin, fluconazole as standard drugs for antibacterial, antifungal agents. MIC was determined by the lowest concentration of sample that prevents the development of turbidity. Antimicrobial activities are shown in Table 1. Compounds showed moderate antimicrobial activity against different strains.

2.5. DPPH free radical scavenging assay:

The effect of **SM-2b, Cu-2b, Cu-3b, and Ni-2b** on DPPH radical scavenging activity was determined according to the method of Yamaguchi et al. [39], with slight modification; vitamin c was used as the reference standard. Briefly, 0.1mmol solutions of DPPH was incubated with 0-100 µM of **SM-2b, Cu-2b, Cu-3b and Ni-2b** for 20 min at ambient temperature in the dark, and the resulting absorbance was measured using UV/Vis spectrophotometer at 517 nm against a blank. The absorbance of DPPH (containing no sample) was recorded as a control. The experiment was done in triplicates. Vitamin C was used as the standard antioxidant. 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 colorimetric method

Direct hemolytic activity was determined using washed human RBC. Briefly, packed human erythrocytes and PBS (1:9 v/v) were mixed; 1 ml of this suspension was incubated independently with the various concentrations

of **SM-2b, Cu-2b, Cu-3b and Ni-2b** (20, 40, 60, 80 and 100 µM/mL) for 1 h at 37°C. The reaction was stopped by adding 9 ml of ice-cold PBS and centrifuged at 1000 g for 10 min at 37°C. The amount of hemoglobin released in the supernatant was measured at 540 nm. The activity was expressed as the percentage of hemolysis against 100% lysis of cells due to the addition of water that served as a positive control, and PBS served as a negative control.

2.7. Molecular Docking study

Ligand 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 [40].

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 [41]. 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 byCHARMm energy and the top scoring (most negative, thus favorable to binding) poses. The binding energy of the compounds was given in Table 2. Binding interaction was given in figs. 7 & 8. Compounds showed good binding energy.

3. RESULTS AND DISCUSSION

All the synthesized ligand and metal complexes are colored amorphous solids. The synthesized ligand and complexes were well characterized by ^{1}H NMR, ^{13}C NMR, Mass, UV, FT-IR, and ESR spectra. There is a very good correlation between theoretical and experimental data.

^{1}H NMR (400 MHz, d6-DMSO): δ 8.377 (s, 2H, $H^{B3,5}$), 7.927-7.935 (d, 2H, J = 3.2 Hz, H^{A2}), 7.454-7.461 (d, 2H, J =2.8 Hz, H^{A3}), 7.438 (s, 1H, ArH), 6.596-6.602 (d, 1H, ArH), 6.565-6.575 (d, 1H, ArH), 3.865 (s, 3H, OCH_3), 3.853 (s, 3H, OCH_3);**^{13}CNMR** (100 MHz, δ ppm): 169.271, 161.828, 157.995, 150.727, 148.801, 143.985, 131.347, 121.482, 120.480, 104.991, 55.693, 55.450.

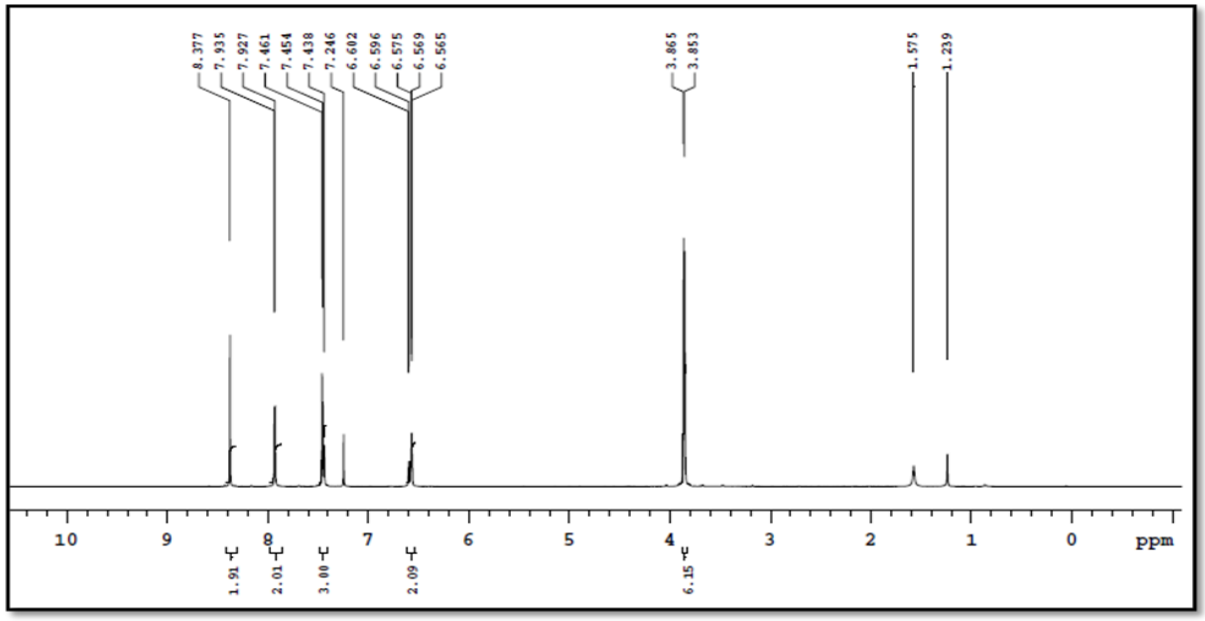


Fig. 1: ^{1}H NMR (400 MHz) spectrum of 2,2'-(4-(2,4-dimethoxyphenyl)pyridine-2,6-diyl)dithiazole (SM-2b) in $CDCl_3$.

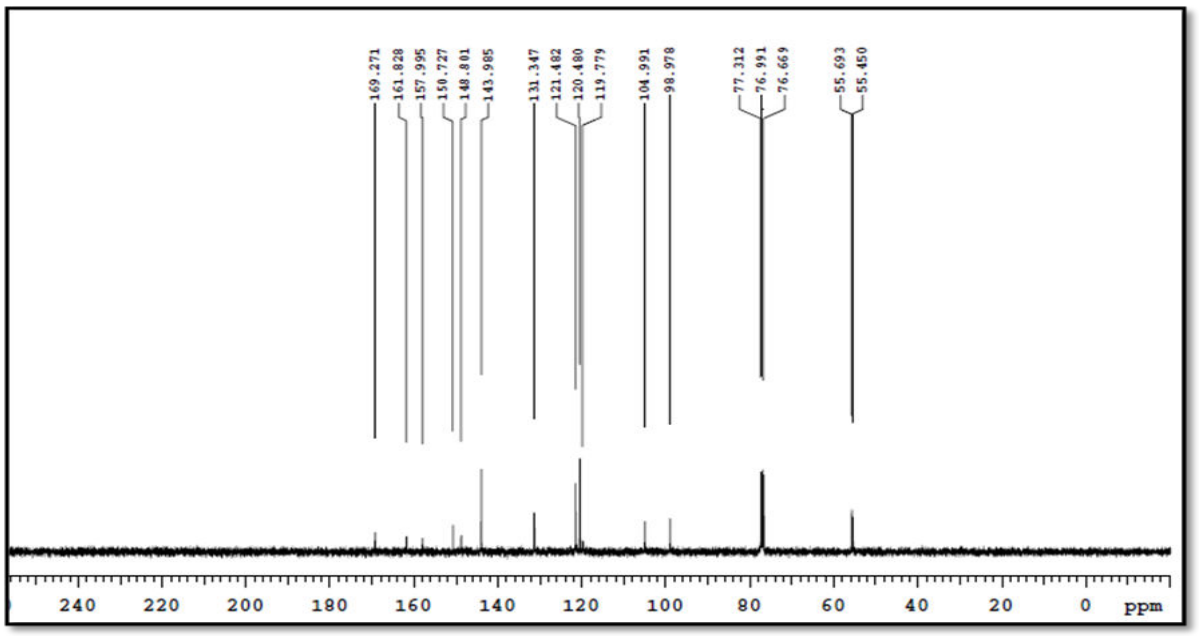


Fig. 2: ^{13}C -NMR (100.6 MHz) spectrum of 2,2'-(4-(2,4-dimethoxyphenyl)pyridine-2,6-diyl)dithiazole (SM-2b) in $CDCl_3$

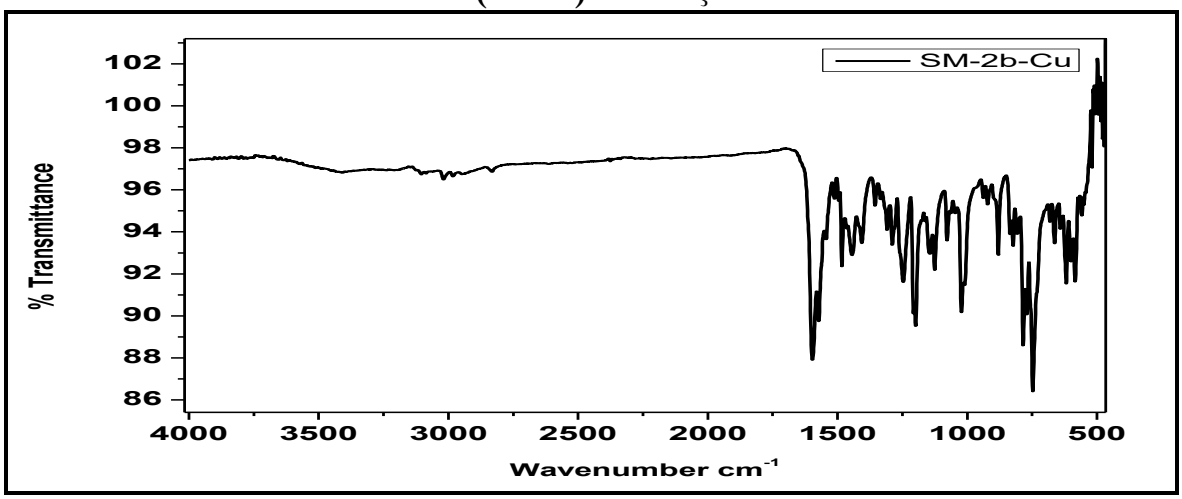


Fig. 3: FT-IR Spectrum of Copper complex

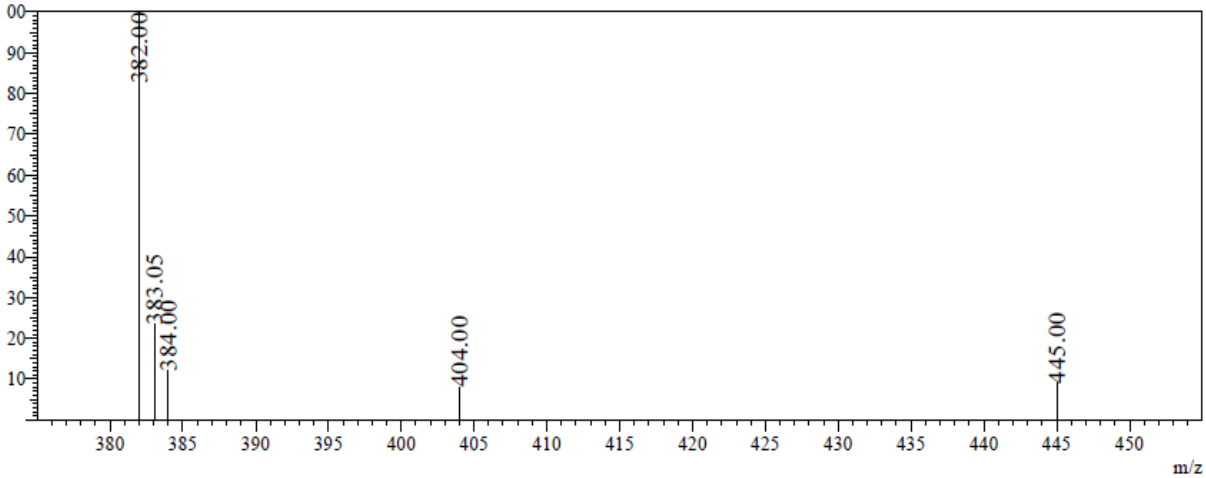


Fig. 4: Mass Spectrum of 2,2'-(4-(2,4-dimethoxyphenyl)pyridine-2,6-diyl)dithiazole

3.1. FTIR Spectrum

The IR spectrum (Fig. 3) exhibits the absorption band at 1688(w) cm^{-1} due to terpyridine stretching, the absorption band at 1127(M) cm^{-1} due to -C-N stretching and 3057 cm^{-1}, 2827 cm^{-1} due to –C-H stretching and 1456 cm^{-1}(s) due to (C-O).

3.2. Mass Spectrum

In the mass spectrum of ligand 2,2'-(4-(2,4-dimethoxyphenyl)pyridine-2,6-diyl)dithiazole the molecular ion was displayed at $M^{+\cdot}$382 which is equal to its molecular weight, this on the loss of hydrogen radical gave fragment ions peak recorded as m/z 381 (100%) which also a base peak as shown in Fig. 4.

3.3. EPR Spectrum

In the EPR spectrum (Fig. 5), the g=2.1785 indicates the presence of free electron and the complex is paramagnetic with distorted octahedral geometry.

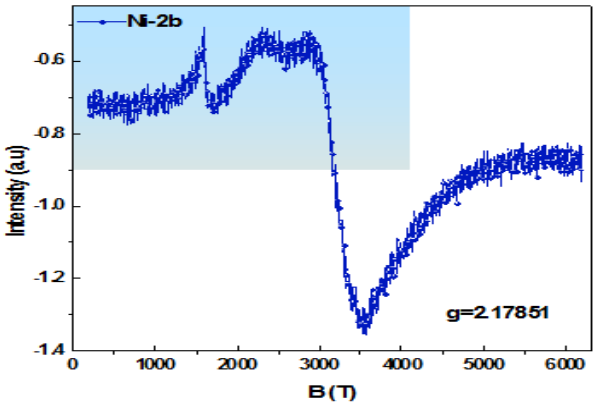


Fig. 5: EPR Spectrum of Ni-2b complex.

3.4. Antibacterial and antifungal assay

All the synthesized compounds showed significant antimicrobial activity against bacterial strains and fungal strains. Interestingly, Copper complexes showed more antimicrobial activity as compared with the ligand and Nickel complexes showed poor antimicrobial activities.

Table 1: Antimicrobial activities of synthesized compounds

Compound	Bacterial strains		Fungal strains	
	S. aureus	*E. coli*	*C. albicans*	*A. niger*
L	8.85	7.08	10.54	9.23
Cu-2a	14.80	13.41	11.38	12.74
Cu-3a	11.56	12.58	10.54	13.20
Ni-2a	7.05	8.24	10.21	9.21
Ciprofloxacin	23.0	23.0	-	-
Fluconazole	-	-	25.0	25.0

3.5. Antioxidant Activity

The antioxidant property of SM-2b, Cu-2b, Cu-3b, and Ni-2b was analyzed by DPPH radical scavenging assay. There was a concentration dependent DPPH radical scavenging activity of compound SM-2b, Cu-2b, Cu-3b, and Ni-2b. At 20-100 µM and there was a significant increase in the antioxidant potential of compound compared with vitamin C (Fig. 6). However, at 100 µM concentrations, the anti-oxidant potential SM-2b, Cu-2b, Cu-3b, and Ni-2b was saturated. The obtained results suggest that compound **SM-2b, Cu-2b, Cu-3b, and Ni-2b** possess very good anti-oxidant activity.

3.6. Direct hemolytic activity

The prepared compounds **SM-2b, Cu-2b, Cu-3b, and Ni-2b** did not hydrolyze red blood cell; it did not cause any effect to human erythrocyte up to the concentration of at 20-100 µM that suggests that the ligand and it's metal complexes possess nontoxic property.

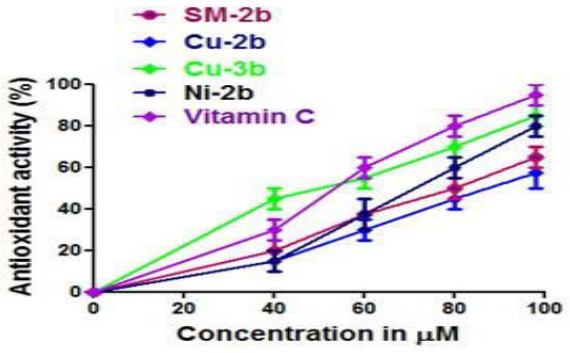


Fig. 6: DPPH scavenging activity of SM-2b, Cu-2b, Cu-3b, Ni-2b

Table 2: Interaction energy values of compounds with target proteins

Compound	C Docker Energy	No of interaction	Interaction residues		
			Pi	Pi-Pi	H bonding
SM-2b	-85.3	11	PRO-770, PHE-771, LEU-694, GLY-695, SER-696		
CU-2b	-105.7	12	LEU-694, GLY-695, SER-696,VAL-702, LYS-704		
CU-3b	-104	14	LEU-694, ALA-719, LYS-721, LYS-721		
Ni-2b	0	-	-		

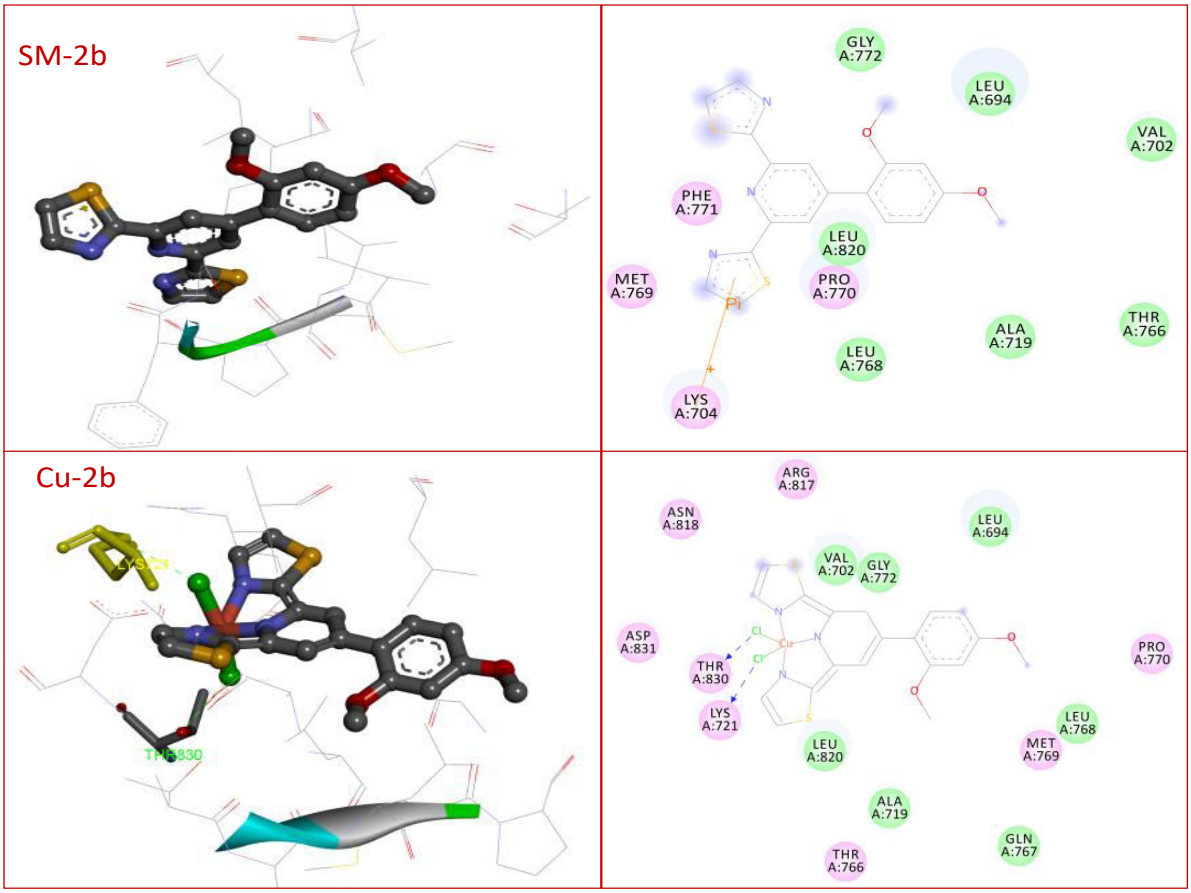


Fig. 7: Binding pattern of SM-2b, Cu-2b with target protein 2XCT

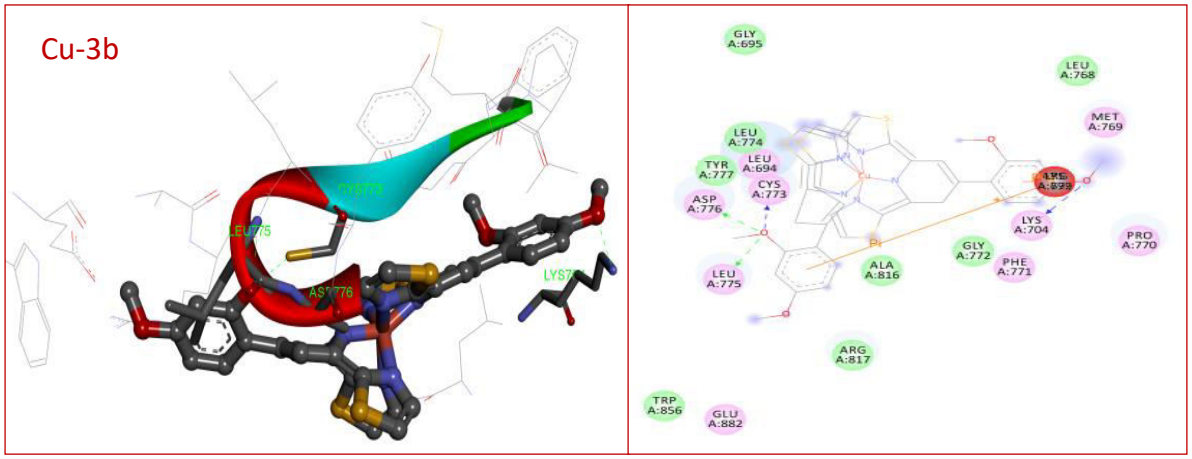


Fig. 8: Binding pattern of Cu-3b with target protein 2XCT

4. CONCLUSION

The novel ligand 2,2'-(4-(2,4-dimethoxyphenyl)pyridine-2,6-diyl)dithiazole and its Copper and Nickel 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, docking and nontoxicity.

5. ACKNOWLEDGEMENT

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