ScienSage

Journal of Advanced Scientific Research

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

ISSN
0976-9595
Research Article

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF 2, 2'-[(6-METHYLPYRIMIDINE-2,4-DIYL)DISULFANEDIYL]BIS(1,3-BENZOTHIAZOLE) AND THEIR CU(II), NI(II) COMPLEXES

Madavi Sunitha*[1], G Ramesh[1], Jayanna Kengaiah[2], Dileep Kumar[1], Divyashree B R[1], Geetha S[1], Shilpa P V[1]

[1]*Department of Studies and Research in Chemistry, Tumkur University, Tumakuru, India*

[2]*Department of Studies and Research in BioChemistry, Tumkur University, India*

**Corresponding author: sanny.iit@gmail.com*

ABSTRACT

In the present study, we reported a novel (NNN) type ligand 2,2'-[(6-methylpyrimidine-2,4-diyl)disulfanediyl]bis(1,3-benzothiazole) and its Cu(II) and Ni(II) complexes. The synthesized complex were characterized by ^{1}H NMR, ^{13}C NMR and Mass spectra. The compounds were screened for antioxidant activity by the percentage of inhibition of 1, 1-diphenyl-2-picryl hydrazyl (DPPH) and compounds found to be potent antioxidants. The prepared compounds were also tested for hemolytic activity. The 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, Complexes, Antioxidant, Hemolysis, Non-toxic.

1. INTRODUCTION

2-mercaptobenzohiazoles and its derivatives possess spectrum of biological and medicinal properties, such as antimicrobial, anti-inflammatory, antioxidant, anti-fungal, antidiabetic, anticancer, antitumor, anticonvul-sant, antitubercular [1-5] etc. Benzothiazole is an interesting scaffold in organic chemistry due to its various biological activities [6-7]. Many derivatives of 2-mercapto-benzohiazoles were known but their metal complexes have not reported in the literature [8]. Coordination chemistry of N-containing heterocycles is a fascinating field in the research [9]. N-containing heterocycles forms various metal complexes with transition metals and rare earth metals [10]. These metal complexes possess wide variety of medicinal properties as well as interesting photophysical, electronic, magnetic and structural properties [11]. In continuation of our work on novel tridentate ligands and their transition metal complexes, in the present study, we reported novel ligand 2,2'-[(6-methylpyrimidine-2,4-diyl)disulfanediyl]bis (1,3-benzothiazole) and its Cu(II) and Ni(II) complexes. Free radical molecules are generated in the body which is main damaging particles due to stress conditions in our life. These free radicals mainly lead to the progression of many pathological events. Removal of free radical generation is the key event in the progression of many compulsive conditions. Due to oxidative stress, these generated free radicals contribute to the major role in inducing cell mediated death. So research focused to find novel antioxidants. Hence, the synthesized compounds were evaluated for antioxidant properties.

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. 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. Mass spectrum recorded on Mass Spectro-photometer. Elemental analysis was done by conventional methods.

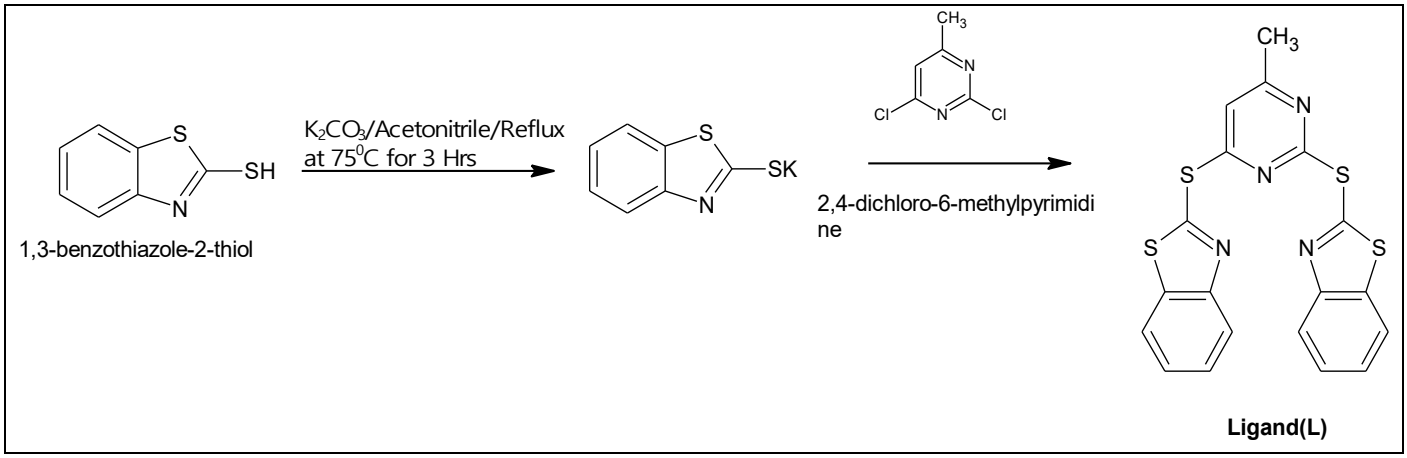
2.2. General procedure

2.2.1. *Preparation of 2,2'-[(6-methylpyrimidine -2,4-diyl)disulfanediyl]bis(1,3-benzothiazole) (L)*

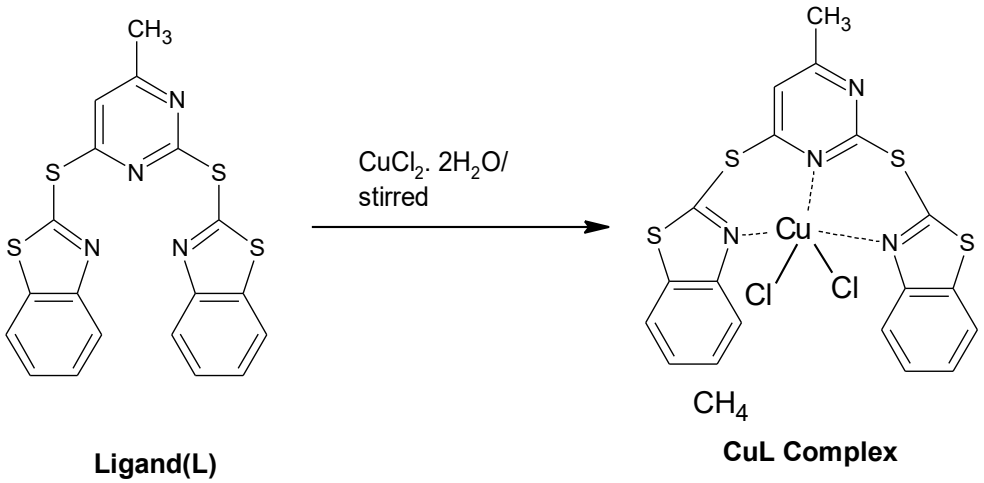
One mole (1.023g) of 2-Mercaptobenzothiazole was dissolvedin acetonitrile solvent and taken in a round bot tom flas, to this solution 0.8458 (1.0 mol) of dried potassium carbonate was added. The reaction mixture was refluxed at 75°C for about 3hrs. After conversion of 2-Mercaptobenzothiazole into potassium 1, 3-benzothia-zole-2-thiolate, 0.5g of 2, 6-dichloro-6-methylpyrimi-dine dissolved in acetonitrile was added drop wise for 30 minutes. The resultant reaction mixture was further refluxed for 12hrs. Reaction progress and completion were monitored by TLC. After completion of reaction,the reaction mixture was taken in a beaker and chloroform was added, then that solution was taken in a separating funnel. The chloroform layer was collected in a beaker, washed with water thrice and finally with the brine solution. To this solution, sodium sulphate was added to remove the water content/moisture, and then it was filtered and evaporated on a rotary evaporator, obtained a brown colored liquid, then it was dried to obtain yellow coloured solid ligand(L) [12].

2.2.2. *Preparation of Copper metal complexes [CuL] (M:L=1:1)*

$CuCl_2.6H_2O$ (100mg; 0.587mmol) was dissolved in 10 ml of methanol taken in a round bottom flask and stirred. Then to this clear solution, 0.58 mmol (249mg) of ligand (**L)** dissolved in chloroform was added dropwise. The reaction mixture was stirred at room temperature for about 12hrs. The progress of the reaction was monitored by TLC using pet ether and ethyl acetate (70:30 %) as mobile phase. A green precipitate obtained was filtered and collected [13].



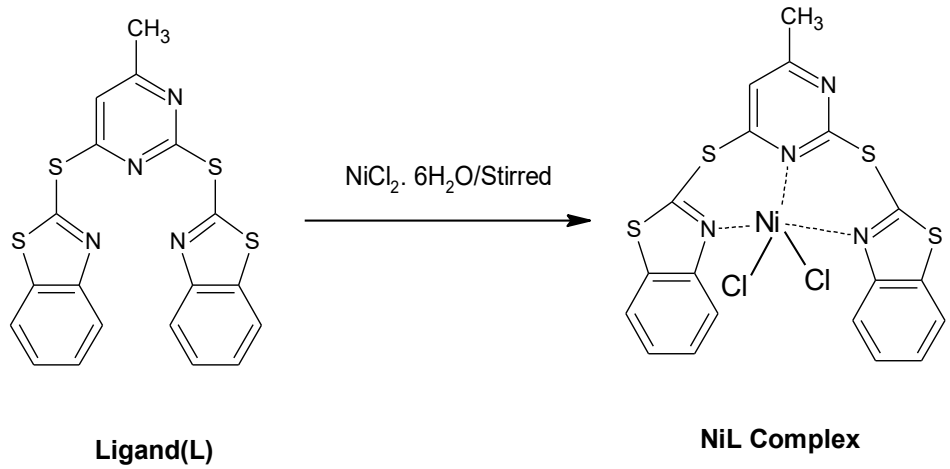
Scheme 1: Synthesis of 2,2'-[(6-methylpyrimidine-2,4-diyl)disulfanediyl]bis(1,3-benzothiazole) (L)



Scheme 2: Synthesis of Copper Complex (CuL)

2.2.3. *Preparation of Nickel metal complex [NiL]: (M:L=1:1)*

$NiCl_2.6H_2O$ (100mg; 0.2103mmol) was dissolved in 10 ml of methanol in a round bottom flask and stirred. Then to this solution, 0.02103mmol(0.893mg) of ligand (L) dissolved in chloroform was added drop wisely. The reaction mixture was stirred at room temperature for about 12hrs. The progress of the reaction was monitored by TLC using pet ether and ethyl acetate (70:30 %) as mobile phase. After 12hrs brown precipitate obtained was filtered and collected [14].



Ligand(L) **NiL Complex**

Scheme 3: Synthesis of Nickel Complex (NiL)

2.3. Antioxidant activity

2.3.1. *Evaluation of antioxidant activity by DPPH radical scavenging method*

Free radical scavenging activity of different synthetic compounds **CuL, L and NiL** synthetic compounds were measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH) method. In brief, 0.1 mM solution of DPPH in ethanol was prepared. This **CuL, L and Ni-L** was added to 3 ml of different synthetic compounds at different concentration (0-100 µm/ml). Here, only those compounds are used which are soluble in DMSO and their various concentrations were prepared by dilution method. The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes. The absorbance was measured at 517 nm using spectrophotometer (UV-VIS thermo scientific). Reference standard compound being used was vitamin C and experiment was done in triplicate. Lower absorbance of the reaction mixture indicated higher free radical activity [15]. The percentage of free radical scavenging was calculated using this formula.

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

2.4. Direct hemolytic activity

Direct hemolytic activity was determined by using washed human erythrocytes. Briefly, packed human erythrocytes and Phosphate Buffer Saline (PBS) (1:9v/v) were mixed; 1ml of this suspension was incubated independently with the various concentrations of **CuL, L and NiL** (0-100 µm) for 1hr at 37°C. The reaction was terminated by adding 9 ml of ice cold PBS and centrifuged at 1000g for 10min at 37°C. The amount of haemoglobin released in the supernatant was measured at 540nm. Activity was expressed as percent of haemolysis against 100% lysis of cells due to the addition of water (positive control), where as PBS served as negative control [16].

3. RESULTS AND DISCUSSION

Ligand 2,2'-[(6-methylpyrimidine-2,4-diyl) disulfane-diyl] bis (1,3-benzothiazole) obtained in good yield 75% by the condensation reaction of 2-Mercaptobenzo-thiazole with 2,4-dichloromethyl pyrimidine. Ligand was characterized by ^{1}H NMR, ^{13}C NMR and Mass spectroscopy. Spectral data obtained confirmed the formation of the ligand. In the **^{1}H NMR** spectrum In this compound **[L]** the proton attached to the phenyl ring (H_6, H_{12}) resonate at 8.040 and 8.020 ppm (d, Ar-H, 2H). The protons of the phenyl ring (H_3, H_{15}) resonate at 7.993 and 7.973 ppm (d, Ar-H, 2H), the three protons of the phenyl ring (H_1, H_2) resonate at 7.578, 7.560 and 7.542 ppm (t, Ar-H, 3H).The three protons of the phenyl ring (H_{10},H_{11}, H_{12}) resonate at

7.494,7.472 and 7.450 ppm (t, Ar-H, 3H). The proton in the pyrimidine ring(H_{21}) resonate at 7.153 ppm (s, Ar-H, 1H). The methyl proton (H_{27}) resonate at 2.479 ppm (s, -CH_3,1H).

In this compound [L] the carbon (C_{26}) appears at 169.154ppm, the C-S (C_{22}) appears at 169.855 ppm, the C=N (C_8,C_{17}) appears at 167.481ppm, the carbon (C_4, C_{14}) appears at 152.430ppm, the carbon (C_5, C_{13}) appears at 137.214ppm,the carbon (C_3, C_{15}) appears at 126.531 ppm,the carbon (C_2, C_{10}) appears at 125.831 ppm, the carbon (C_6, C_{12}) appears at 123.262 ppm, the carbon (C_1,C_{11}) appears at 120.947 ppm, the carbon C_{21}) appears at 114.915 ppm , the (C_{27}) appears at 23.860 ppm respectively.

Ligand Show the molecular peak ion at **m/z 424.8931 confirming** the assigned structure.

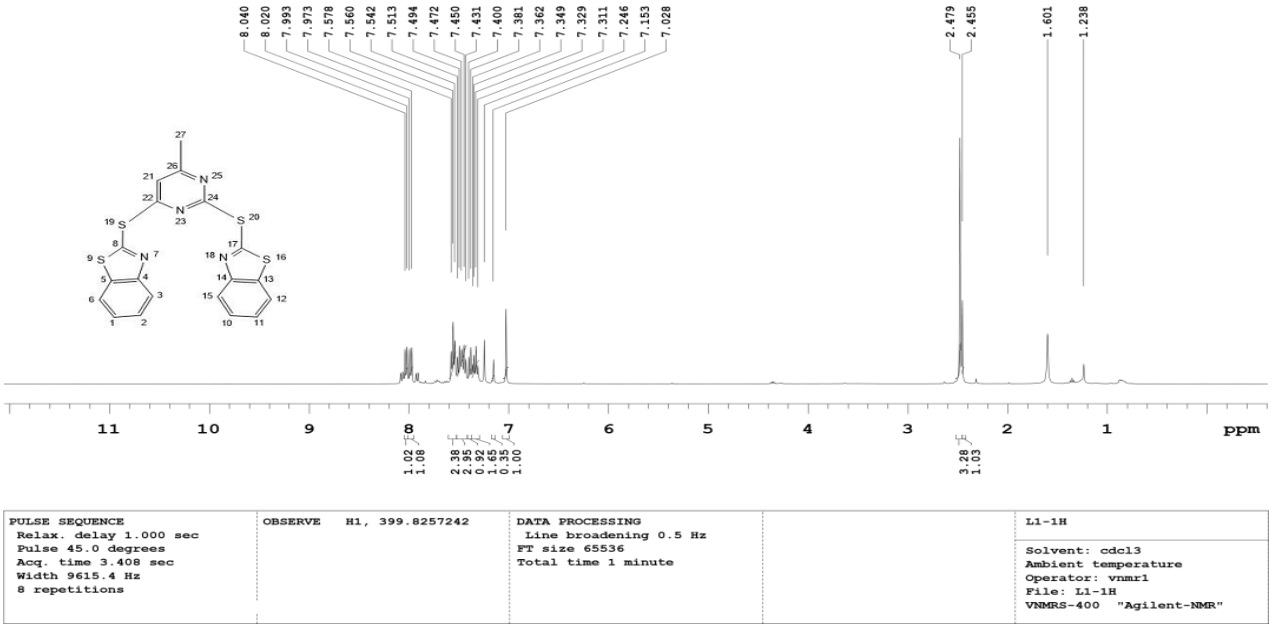


Fig.1: ^{1}H NMR spectrum of 2,2'-[(6-methylpyrimidine-2,4-diyl)disulfanediyl]bis(1,3-benzothiazole) (L) in $CDCl_3$:

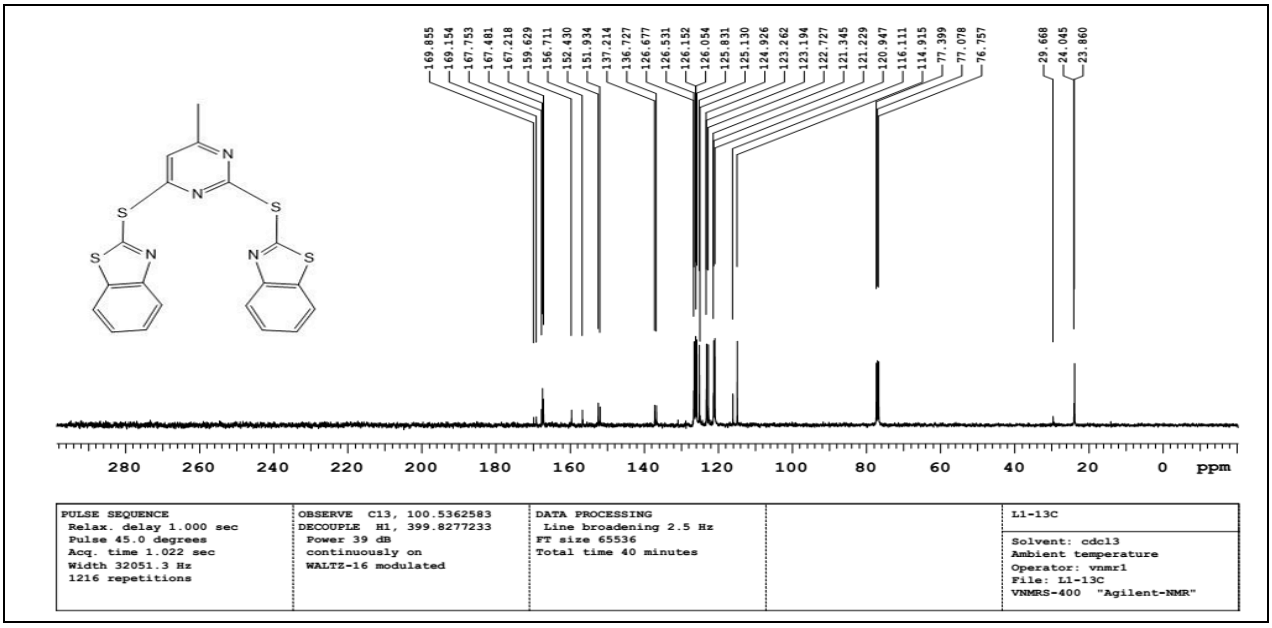


Fig. 2: ^{13}C NMR spectrum of 2,2'-[(6-methylpyrimidine-2,4-diyl)disulfanediyl]bis(1,3-benzothiazole) (L) in $CDCl_3$

3.1. Antioxidant potential of L, Cu-L and Ni-L:

Cu-L, L and Ni-L Synthetic compounds better antioxidant potential when compare to standard vitamin C by DPPH scavenging assay method. The absorbance at 517 nm by UV visible spectrophotometer was found to be as potential for standard vitamin C and synthetic extract respectively. It means synthetic compounds at higher concentration captured more free radicals formed by DPPH resulting into decrease in absorbance.

3.2. Effect of L, Cu-L, and Ni-L on human erythrocyte

PSPE did not hydrolyze RBC up to the concentration of 0-100µM compared with positive control and PBS buffer used as a negative control. Free radical scavenging efficacy of **L, Cu-L and Ni-L** in terms of a DPPH radical scavenging assay vitamin C was used as a standard.

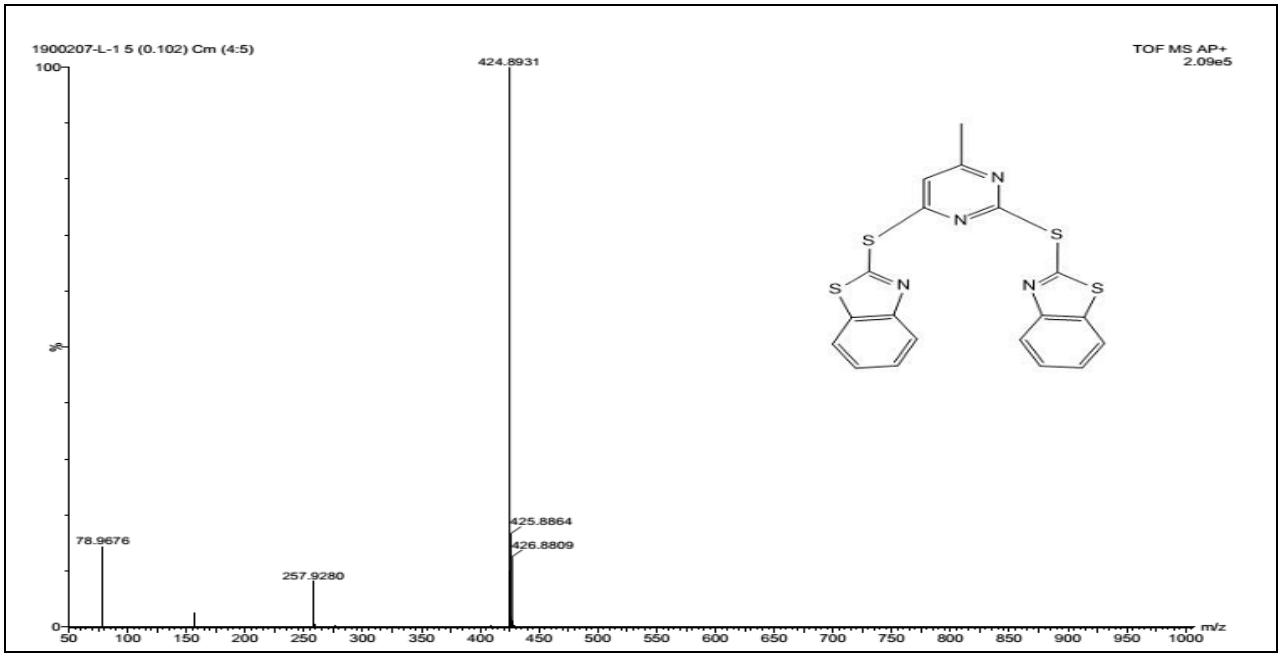


Fig. 3: Mass Spectrum 2,2'-[(6-methylpyrimidine-2,4-diyl)disulfanediyl]bis(1,3-benzothiazole) (L)

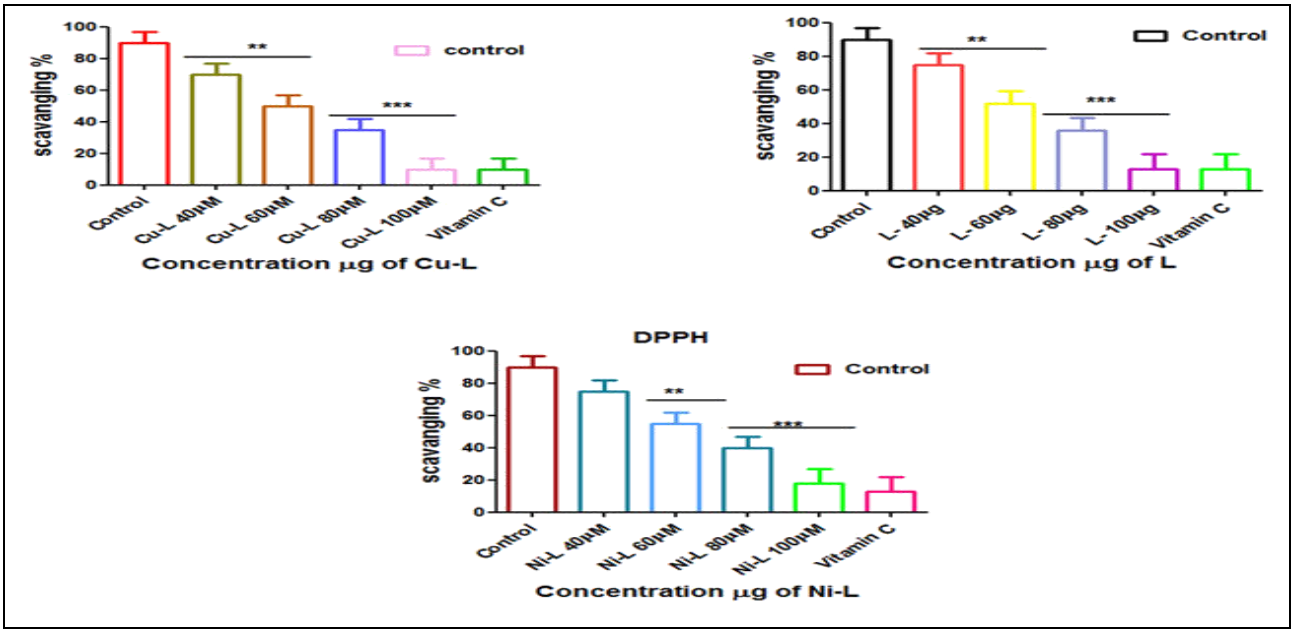
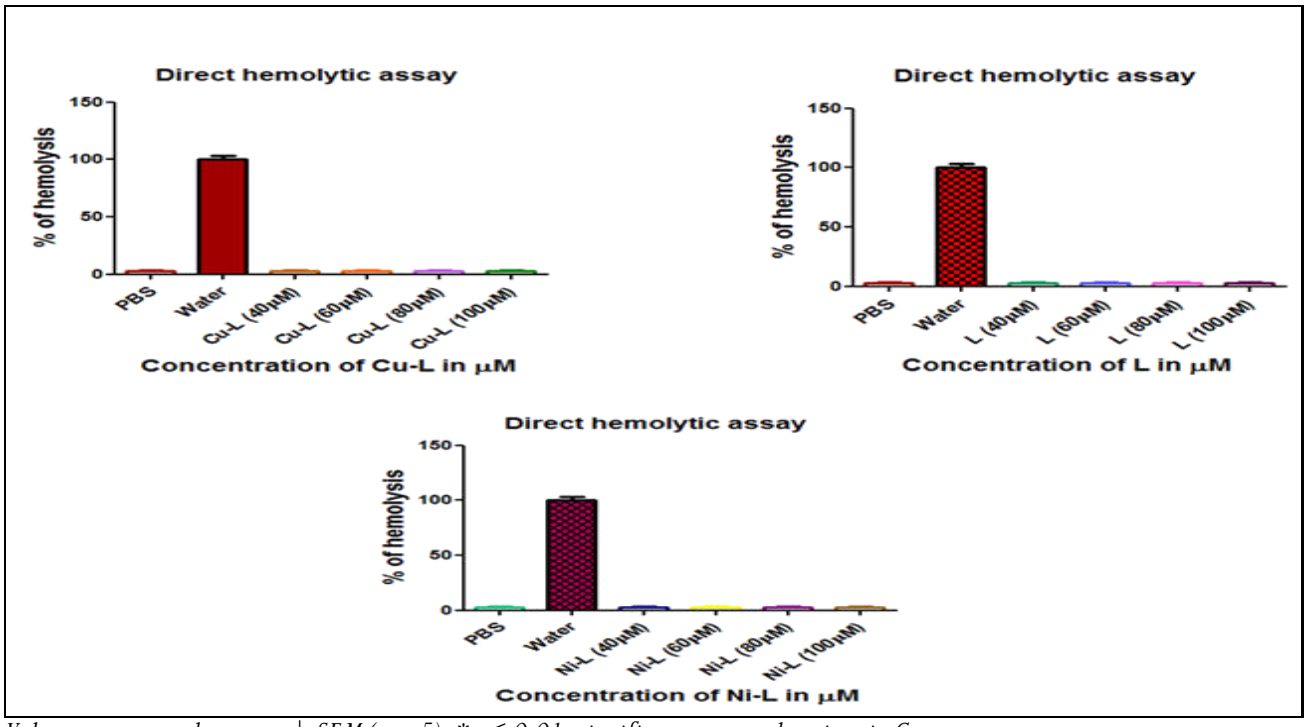


Fig. 4: DPPH scavenging activity



*Values are presented as mean ± SEM (n = 5). *p < 0.01; significant compared to vitamin C.*

Fig. 5: Hemolytic activity of CuL, L and NiL

4. CONCLUSION

The present study was carried out for the synthesis of some effective therapeutics derivatives of 2-Mercapto-benzothiazole with the aim of some pharmacological activities such as antioxidants and hemolytic activities.

New 2-Mercaptobenzothiazole derivative ligands have been synthesized by treatment of 2-mercaptobenzo-thiazole with 2,4 dichloromethyl pyrimidine (L1.Cu(II), Ni(II) complexes have been synthesized. The ligand and complexes were characterized by, ^{1}H NMR, ^{13}C NMR and Mass spectroscopy.

The present study indicates that synthesized 2-Mercaptobenzothiazole derivatives shows increased free radical scavenging activity using the stable radical DPPH.

At 0-80µM there was a remarkable change in the antioxi dant potential of the compound**,**

L, Cu-L and Ni-L when compared with vitamin C. The prepared ligand and complexes exhibited non-toxic property as they did not cause any effect to human erythrocyte.

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 antioxidant and hemolytic activities.

6. REFERENCES

1. D'Amico JJ, Tung CC, Campbell RH, Mullins DD. *J Chem. Eng. Data.* 1963; **8**:446-450.
2. D'Amico JJ, Webster ST, Campbell RH, Twine CE. *J Org Chem.* 1965; **30**:3625-3527.
3. Allen CFH, Rochester NY, Bell A, Knoxville T. *US Patent* 2,450,777, Oct. 5, 1948, Application No.: 581,979, Apr. 20, 1944.
4. Sekar R, Srinivasan M, Marcelis ATM, Sambandam A. *Tetrahedron Lett.* 2011; **52**:3347-3352.
5. Shi L, Liu X, Zhang H, Jiang Y, Ma D. *J. Org. Chem.* 2011; **76**:4200-4204.
6. M Djukic, M Fesatidou, I Xenikakis, A Geronikaki, V. T Angelova, V Savic, M Pavlica, *J. Applicable. Chem.*, 2018; **7(1):**177-188
7. Rahim F, Muhammad TJ, Hayat U, Abdul W, Muhammad T, Muhammad A, et al. *Bio.Org.Chem.,* 2015; **62**:106-116.
8. D'Amico JJ, Harman MW, Cooper RH. *J. Am. Chem. Soc.* 1957; **79**:5270-5276.
9. Morgan KJ. *J. Chem. Soc.* 1958; **166:**854-858.
10. Murru S, Ghosh H, Sahoo SK, Patel BK. *Org Lett.* 2009; **2**:4254-4257.

11. Harizi A, Romdhane A, Mighri Z. *Tetrahedron Lett.* 2000; **41**:5833-5835.
12. Seenaiah DP, Ramachandra Reddy, Mallikarjuna G, Reddy, Padmaja A. *Eur. J. Med. Chem.,* 2014; **77**:1-7.
13. Madavi S, Ramesh G, Venkateshappa G, Jayanna Kengaiah, Shivananda MK. *Int. J. Res. Anal. Rev.*, 2019; **6(1):**681-685.
14. Madavi S, Golla R, Venkateshappa G, Shet PM, Jayanna K, Shivananda MK. *J. Applicable. Chem.*, 2019; **8 (3):**1213-1222.
15. Yamaguchi T, Takamura H, Matoba T, Terao J. *Biosci. Biotechnol. Bio Chem.*, 1998; **62**:1201-1204.
16. Madavi S, Jyothi KS, Megha SB, Azgar P, Golla R, Shivananda MK. *J. Applicable. Chem.*, 2019; **8(2):**654-660.
17. Jayanna K, Sharath Kumar MN, Chethana R, Chandramma, Ashwini S, Devaraja S, *Asian. J. Phar. Pharmac,* 2019; **5(5**):61-65.