Journal of Applied Pharmaceutical Science Vol. 4 (12), pp. 095-101, December, 2014
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2014.41217
ISSN 2231-3354

Synthesis, spectroscopic characterization and antimicrobial activity studies of 2-Butyl-4-chloro-5-formylimidazole thiosemicarbazone and its manganese (II) complex

A. Sreenath Reddy[1] , M. Sivasankar Reddy[1], Venkata Subbaiah kotakadi[2], P.V. Chalapathi[3], A. Varada Reddy[1]*

[1] Department of Chemistry, Sri Venkateswara University, Tiupati-517502, A.P., India.
[2] DST-PURSE Centre, Sri Venkateswara University, Tirupati-517502, A. P., India.
[3] Department of Chemistry, Sri Venkateswara Arts College (TTD), Tirupati-517501 ,A. P., India

ARTICLE INFO

Article history:
Received on: 17/10/2014
Revised on: 06/11/2014
Accepted on: 23/11/2014
Available online: 29/12/2014

Key words:
Thiosemicarbazones; Manganese(II) complex; EPR spectra; Antimicrobial activity.

ABSTRACT

A novel chelating tridentate organic ligand, 2-Butyl-4-chloro-5-formylimidazole thiosemicarbazone(L) is synthesized and characterized by using spectroscopic techniques like elemental analysis, FT-IR, ^{1}H and ^{13}C NMR, UV-vis and Mass spectrometry. The free ligand is then used for the synthesis of Mn(II) complex thoroughly characterized by FT-IR, EPR and electronic spectral analysis. The complex is found to have characteristic d^5 electronic spectrum and the geometry of the complex is identified as octahedron based on the g value obtained from the EPR spectrum. Both the ligand and $Mn(II)L_2$ compounds are found to exhibit similar antimicrobial activity against the gram –ve and gram +ve bacteria.

INTRODUCTION

Among the several existing chelating reagents, thiosemicarbazones have occupied a respectable position in the transition metal chemistry because of their biological prominence. Oflate the thiosemicarbazones and their metal complexes were found to exhibit moderate to good activity against tumor cell lines (Jagadeesh *et al*., 2014; Offiong and Martelli, 1997), and pathogenic bacteria (Prathima *et al*., 2011; zhu Y.K *et al.,* 2011) as anti-oxidants (Subba Rao *et al*., 2010; Choudhary *et al*., 2011), anti-convulsant (Hemlata *et al*., 2010), anti-malarial (Chipeleme *et al*., 2007), anti-diabetic (Naveen *et al*., 2012), anti-proliferative (Ana *et al*., 2001), anti-tuberculosis (Sanjay *et al*., 2014), anti-inflammatory (Gonzalez *et al*., 2009) and many other biological applications (Matesanz and Souza, 2007; Mendes *et al*., 2006). Especially first row transition metal complexes of thiosemicarbazones have extensive range of biological activity (Chandra and Anil Kumar, 2007). Sulphur and nitrogen both being the donor atoms in the case of thiosemicarbazone are found to be responsible for appreciable biological activity in different fields.

Normally, transition metal ions when coordinated to the organic ligands can increase their lipophilic character thus allowing their interaction with the cellular constituents effectively. Hence, thiosemicarbazone metal complexes are known to exhibit better activity than their corresponding free ligands and other organic molecules (Prathima *et al*., 2012).

In the present work, the author mainly concentrated on the synthesis and spectroscopic characterization of the novel imidazole thiosemicarbazone ligand and its manganese(II) complex. The compounds are further tested for antimicrobial activity against gram negative and gram positive pathogens.

* *Corresponding Author*
Email: ammireddyv@yahoo.co.in

Experimental

Chemicals

All the chemicals used in the present work were of analytical grade. The organic solvents were obtained from the commercial sources and used without further purification. 2-Butyl-4-chloro-5-formylimidazole (BCFI) was purchased from Sigma Aldrich, thiosemicarbazide and Manganese(II) chloride were purchased from Sd-Fine chemicals.

Instrumentation

A shimadzu 2450 UV-vis spectrophotometer equipped with a 1.0 cm quartz cell is used for the absorbance studies. Elemental analysis (CHN) were performed using SEM attached Inca Penta FETX3 Oxford instruments. Infrared spectra of the compounds are recorded on a Bruker Alpha- Eco ATR–FTIR (Attenuated total reflection–Fourier transform infrared) interferometer with single reflection sampling module equipped with ZnSe crystal. The NMR spectrum of L is recorded on a Bruker (400MHz) spectrometer at 300 ºK, using DMSO-d^6 as a solvent and tetramethylsilane (TMS) as an internal reference compound. Mass spectra of the free ligand and its Mn(II) complex are recorded in ESI mode using a Bruker HRMS stationed at University of Hyderabad. EPR spectra are recorded as poly crystalline sample at 298 ºK an a Bruker-ERO 73 instrument equipped with an EMX microX Source for X band measurement using Xenon 1.15.60 Software provided by the manufacturer.

Synthesis of 2-Butyl-4-chloro-5-formylimidazole thiosemicarbazone (L)

To a methanolic solution of 2-Butyl-4-chloro-5-formylimidazole (0.01 mole; 1.87 gm) is added thiosemicarbazide (0.01 moles; 0.92 gm) under vigorous stirring. The reaction mixture is refluxed for about 3-4 h by checking the progress of the reaction using TLC technique. The precipitate is collected by filtration and recrystallized using ethanol. The proposed structure can be shown according to **scheme. 1.** Light Yellow coloured crystals, yield: 71%. Elemental analysis(%) for $C_9H_{14}N_5SCl$: calc. C, 41.61; H, 5.43; N, 26.96; found. C, 41.52, H, 5.41, N, 26.85; HRMS (m/z): 260 $[C_9H_{14}N_5SCl]^+$. IR (cm^{-1}): 3248 s, (ν_{asy} NH_2 and ν NH); 1609 s, $\delta(NH_2)$+ ν(C=N), 1231 m & 787 s, ν(C=S); NMR spectrum (Bruker, 400 MHz, DMSO- d^6, ppm): ^{1}H-NMR; δ = 0.89 (3H, t, CH_3), 1.29-1.33 (2H, m, CH_2), 1.61-1.64 (2H, m, CH_2), 2.61 (2H, t, CH_2), 7.82 (1H, s, CH), 7.94 (1H, s, NH), 8.32 (1H, s, NH), 11.47 (1H, s, NH), 12.36 (1H, s, NH); ^{13}C-NMR; δ = 178.2(C1), 129.9 (C2), 122.0 (C3), 129.1 (C4), 150.4 (C5), 28.2 (C6), 30.1 (C7), 22.1 (C8), 14.0 (C9).

Synthesis of Mn(II)L_2 complex

To an ethanolic solution of the **L** (0.001 moles; 0.259 gm), $MnCl_2$. $4H_2O$ (0.0005 moles; 0.099 gm) dissolved in aqueous ethanol is added under stirring and the contents of the round bottomed flask was refluxed for 5 h. Yellow coloured solid formed was collected after filtration and washings with hot ethanol. The residue was then dried in a desiccator. Yellowish coloured precipitate, yield: 59%. Elemental analysis(%) for $[Mn(C_9H_{14}N_5SCl)_2]$: calc. C, 37.76; H, 4.81; N, 24.45; found. C, 37.63, H, 4.91, N, 24.38: HRMS (m/z): 572.06 $[Mn(C_9H_{14}N_5SCl)_2]^+$ ion. IR (cm^{-1}): 3250 br, 3167 s, (ν_{as} NH_2 and ν NH); 1605 s, $\delta(NH_2)$ + ν(C=N); 1242 m, 811 m, ν(C=S); 472 m (ν Mn-N).

Antimicrobial activity

The synthesized ligand and its manganese complex are subjected for in-vitro antimicrobial assay against two gram negative antibiotic resistant Escherichia coli strains (Mutant and Recipient) and gram-positive Bacillus megaterium, Staphylococcus aureus were used in the present study by the agar disc diffusion method. (Asiri and Khan, 2010) The pathogens are sub cultured in nutrient agar medium by incubating at 37 ^{o}C for 24 hours.

A bacterial suspension of about 10^{-5} CFU/mL is mixed and poured on to the agar medium in the agar plate maintained at RT in a laminar flow cabinet. The minimum inhibitory concentrations of the two synthesized compounds are predicted by preparing different concentrations (30 mcg and 60 mcg) by serial dilution of test samples that are previously dissolved in 0.1 percent DMSO. Filter paper discs of about 6.0 mm in diameter are soaked in the sample of varying concentration and are fixed on to the nutrient agar medium. Amoxiclav (30 mcg) is used as positive control and filter discs wetted with 0.1 percent DMSO are used as negative control. The result obtained from antimicrobial activity is assed after incubating the sample for about 24 hours at 37 ^{o}C. Each experiment is performed in duplicate.

Results and discussion

Infrared spectral analysis

The free ligand exhibited significant vibrational bands corresponding to the important functional groups in the synthesized molecules. The formyl group on the imidazole ring has lost its identity after subjected for condensation with the thiosemicarbazide. A clear cut evidence of the formation of the imine (C=N) functionality is found with the strong and sharp stretching vibrational band at 1609 cm^{-1} coupled with the N-H bond bending vibrations of the free ligand which further on coordination to the metal ion has shown a negative shift towards 1605 cm^{-1}.

The C=N bond in the imidazole ring is characterized by a sharp peak at around 1557 cm^{-1} (Leovac *et al*., 2007) in the case of the free ligand. The thione (C=S) function of the ligand has shown a sharp intense peak at 1068 cm^{-1} which has shown some characteristic shift (towards 1062 cm^{-1}) after coordination to the Mn(II) ion. The observed variation of shift after coordination is thought to be because of the neutral form of the thione group (Krishnan *et al*., 2010). The two donor atoms on bonding with the Mn(II) ion resulted in the decrease of bond order between the C=N and C=S bonds which stands as a characteristic sign for the formation of the complex. **Fig. 1.0.** shows the IR spectral data of the free ligand and it Mn(II) complex.

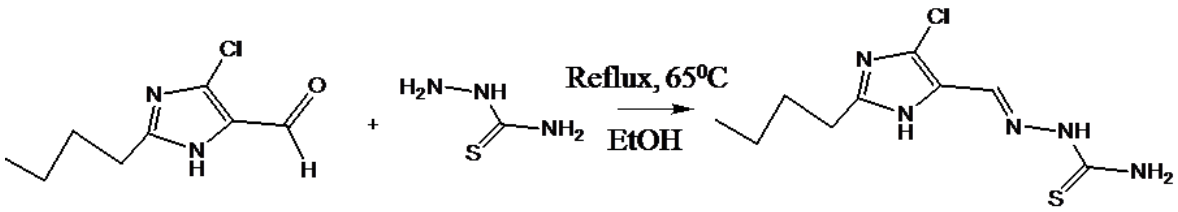

Fig. 1: Schematic representation of synthesis of ligand)

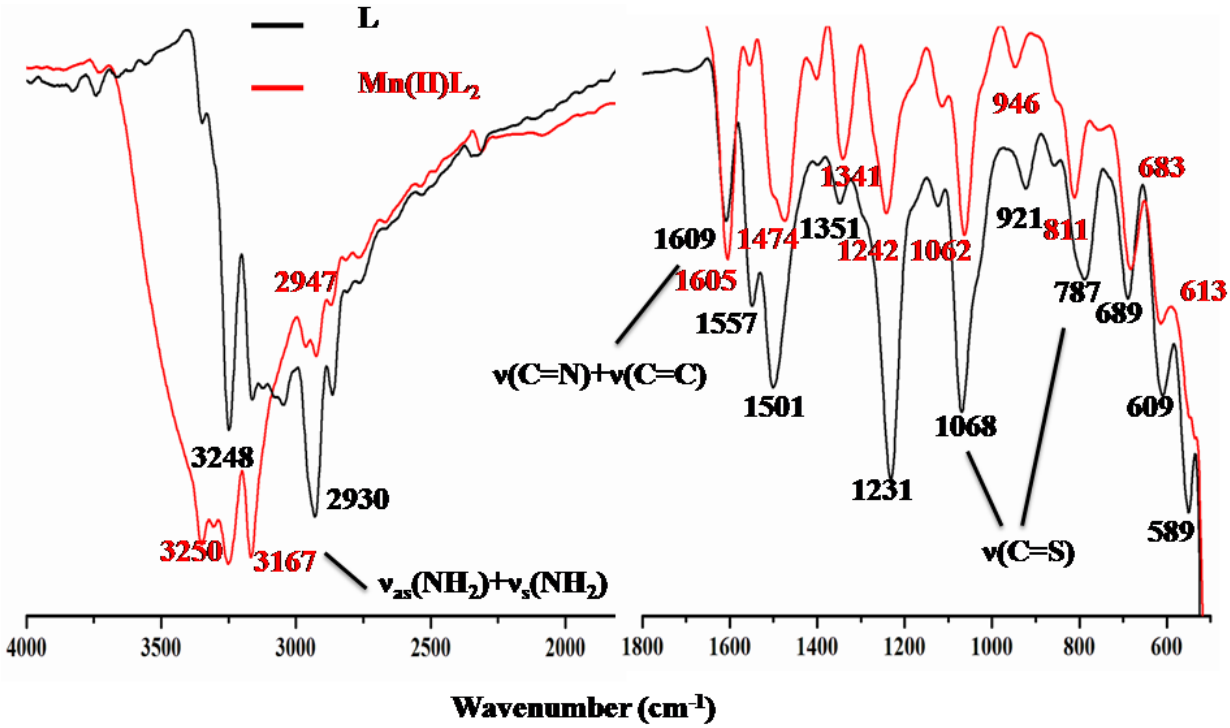

Fig. 2: (IR spectra of the Ligand and its Mn(II) complex)

NMR spectral analysis

The ^{1}H NMR spectrum of the synthesized ligand stood as the important tool to identify the structure of the molecule. The amino protons have shown the chemical shift values of δ 7.94 and 7.82 as two different singlets due to of the involvement of one proton in hydrogen bonding with the imine nitrogen. Two different secondary amine protons are observed as singlets each at δ 11.4 and 12.3 ppm respectively for the N-H present in between the thione and imine functions and N-H of the imidazole ring. The aliphatic protons on the n-butyl side chain attached to the imidazole ring in a row are found at the chemical shifts δ2.61 (t, 2H attached to C6 carbon), 1.62 and 1.32 for the protons attached to C7 and C8 carbons respectively. The methyl protons (C9) are found as triplet at δ 0.89 ppm.

From the ^{13}C NMR analysis of free ligand the environment of the carbon atoms was identified. The thione carbon has shown a peak at δ178.2 ppm where as the imine carbon was found at δ 129.9 ppm. The imidazole ring carbon atoms in a row starting from the aldehydic carbon are found at δ129.1 (C3), 122.0 (C4, attached to chlorine) and at δ 150.4 (C5) ppm respectively. The n-butyl chain attached to imidazole ring has shown the chemical shift values of δ 28.2, 30.1, 22.1 and 14.0 ppm respectively for C6, C7, C8 and C9 carbon atoms. **Fig. 2.1 and 2.2**. shows the ^{1}H and ^{13}C NMR spectral data for the free ligand.

Electronic spectra

The UV-visible spectrum of the free ligand has potentially shown a allowed band corresponding to the π→π* transition as an intense peak at 330 nm. The conjugation of the double bonds in the imidazole ring has allowed the band to experience bathochromic shift towards lower wavelengths. A shoulder like peak partially overlapped by the π→π* band is corresponds for the most probable forbidden n→π* transition. The electronic spectrum of the Mn(II) complex in DMSO is recorded from 400 nm to 650 nm. The complex has shown a typical electronic spectral bands for the Mn(II) ion in an octahedral environment. The electronic spectrum of the complex is shown in the **Fig. 3.2**.

The charge transfer from the ligand has dominated the d-d bands of the Mn(II) ion significantly because of their spin and laporte forbidden character as the usual transitions of the d^5 metal ion in high spin conditions are between different spin energy levels, the transitions with an ε max of 0.01-0.03 are only observed.

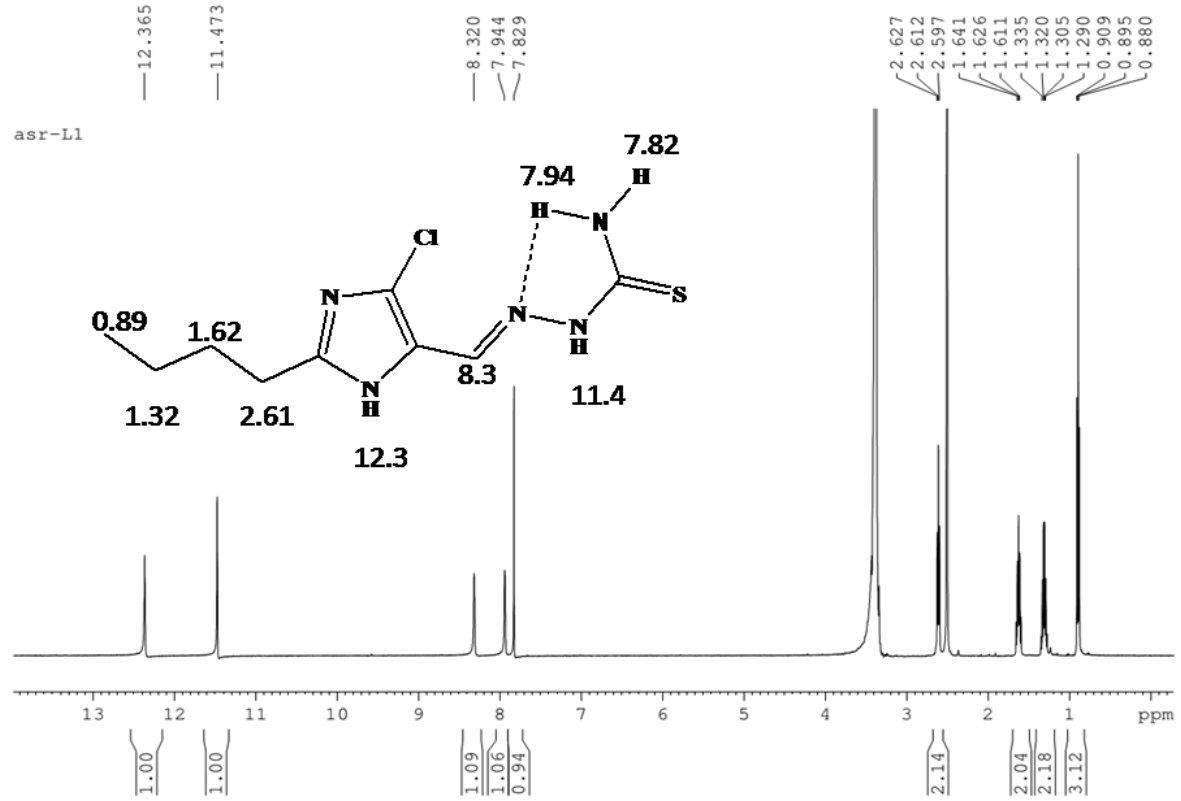

Fig. 3: (1H nmr spectrum of the synthesized ligand)

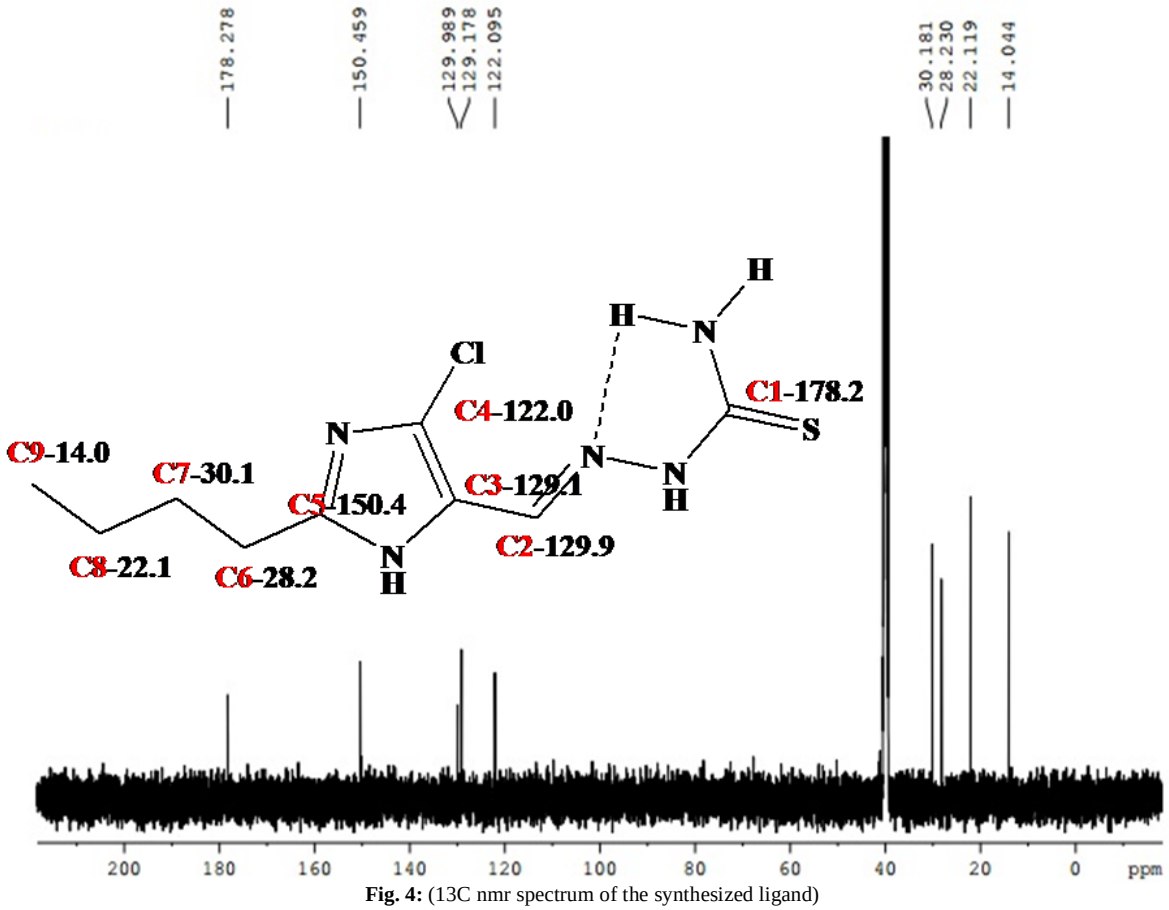

Fig. 4: (13C nmr spectrum of the synthesized ligand)

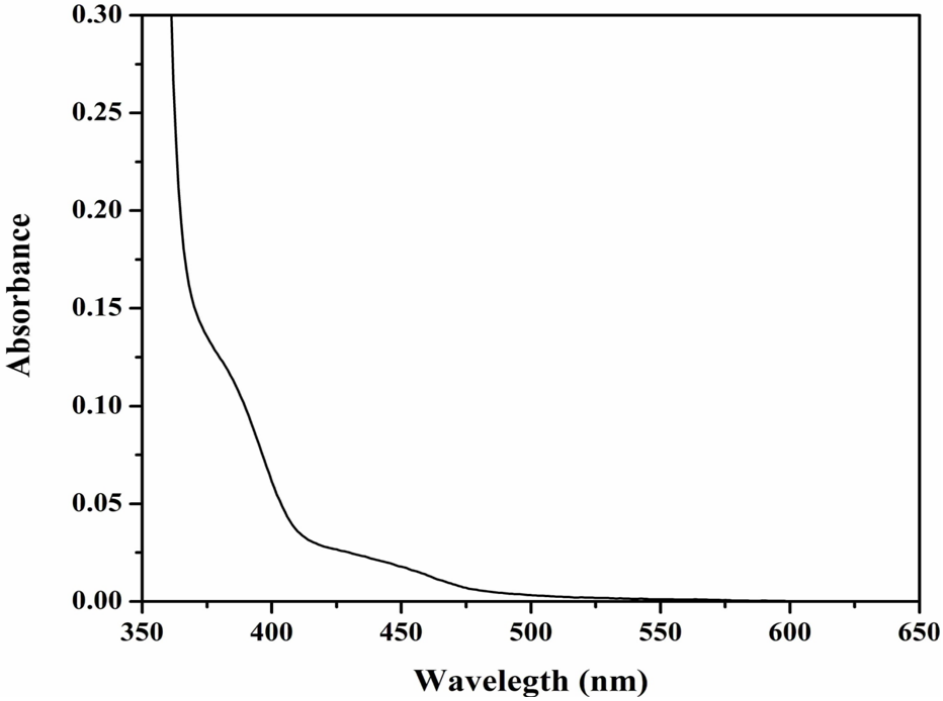

Fig. 5: (Electonic spectrum of the Mn(II) complex)

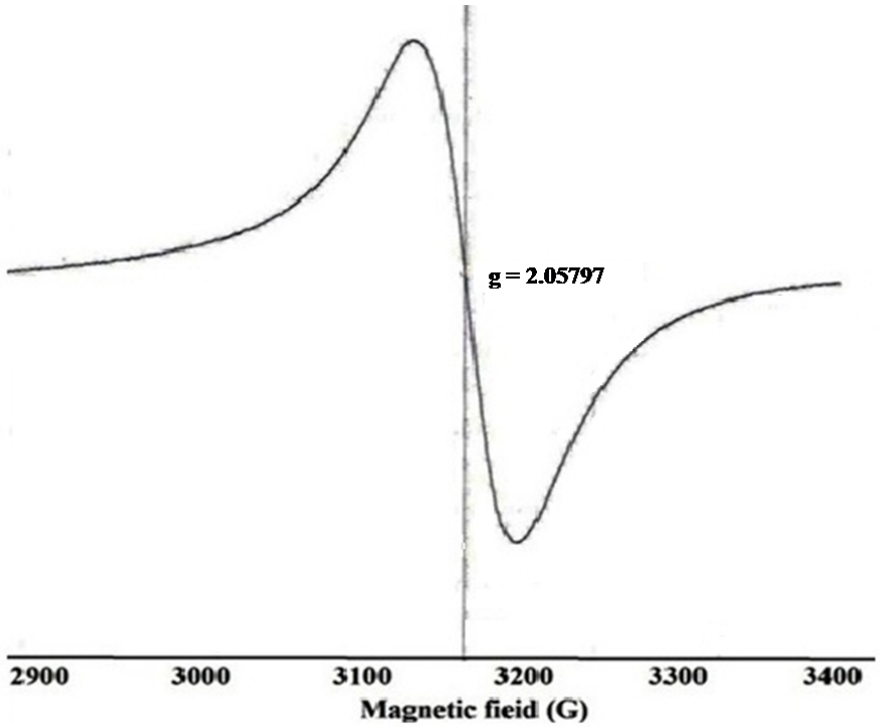

Fig. 6: (EPR specta of the Mn(II) complex)

Table. 1: (Antibacterial screening data of the ligand and its Mn(II) complex)

Composition	Conc, of comp per disc(mcg)	Gram negative bactrria		Gram positive bactrria	
		E. coli (mutent)	E. coli (recipicnt)	Bacillus megaterium	Staphylocoeens surens
		Zone of inhibition in mm (mean of three replicates)			
Ligand	30	7	7	8	10
	60	15	14	17	19
Mn(L)2	30	8	8	8	10
	60	16	15	16	18
amoxclar	30	12	13	12	11

Table 2: (MIC values of the ligand and its Mn(II) complex)

Composition	Gram negative bacteria		Gram positive bacteria	
	E. coli (mutent)	E. coli (recipient)	Bacillus megaterium	Staphylococcus aurens
Ligand	5	10	10	5
Mn(L)2	5	10	10	5

Electron paramagnetic spectra

The EPR spectra of the $Mn(II)L_2$ complex is recorded as polycrystalline sample in Bruker-ERO 73 instrument equipped with an EMX microX Source for X band measurement spectrometer by using DPPH as the standard. A single non resolved broad spectrum obtained for the complex at room temperature is attributed for the restricted rotations of the molecule in solid state and due to the negligible levels of zero field splitting (Krishnan *et al*., 2010).

Fig. 4.0. shows the EPR spectrum of the manganese complex. The g-value was found to be 2.0579 which is slightly greater than the g value of the free electron (g=2.023) suggesting octahedron geometry (Chandra and Lokesh Kumar, 2005).

Antimicrobial activity

The synthesized compounds are subjected for the antimicrobial activity assay against some human pathogenic microorganisms like *E. coli*, *B. megaterium* and *S. aureus* by using agar disc diffusion method. Both the free ligand and its Mn(II) complex has shown almost similar behavior with the gram +ve and gram –ve bacteria. **Table 1.** shows the inhibition zones formed on the agar plates after incubation at 37 ^{o}C for 24 hours.

Determination of minimum inhibitory concentration(MIC)

The minimum inhibitory concentration (MIC) is the lowest concentration of visible growth after over night incubation. Minimum inhibitory concentration are important in diagnostic laboratories to conform resistant of microorganisms to antimicrobial agents and also to monitor the activity of the new microbial agents. MIC measurement was performed using a modified agar well diffusion method. **Table 2.** shows the minimum inhibition zone values.

CONCLUSIONS

A strong coordination is found between the tridentate 2-Butyl-4-chloro-5-formylimidazole thiosemicarbazone ligand and the manganese(II) ion from the IR spectal data. The ligand acted as uninegative molecule in basic medium to satisfy the primary and secondary valancy of the manganese(II) ion. A distorted octahedral geometry is assigned for the complex form the EPR calculations. Finally, both the free ligand and its manganese(II) complex were found to show only moderate antimicrobial activity against the human pathogens when compared to the standard amoxiclav.

ACKNOWLEDGEMENTS

The author Mr A. Sreenath Reddy, is thankful to UGC-BSR New Delhi for providing financial support in the form of Meritorious Fellowship under RFSMS scheme and UGC Networking Resource Centre, University of Hyderabad, Hyderabad, India for providing space to carry out the characterization of the compounds. The author also expresses his heartfelt thanks to **Prof. Samar K. Das** for his help in carrying out the research work in his laboratory**.**

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How to cite this article:

Sreenath Reddy A., Sivasankar Reddy M., Venkata Subbaiah kotakadi, Chalapathi P.V., Varada Reddy A. Synthesis, spectroscopic characterization and antimicrobial activity studies of 2-Butyl-4-chloro-5-formylimidazole thiosemicarbazone and its manganese(II) complex. J App Pharm Sci, 2014; 4 (12): 095-101.