Available on line www.jocpr.com

Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2010, 2(1): 292-299

ISSN No: 0975-7384

Synthesis, characterization and bioactivities of Ni(II) and Co(II) complexes of benzyloxybenzaldehydethiosemicarbazone

Y. Subba Rao[a], B. Prathima[a], O. Hariprasad[b], N. Nagabhusan Reddy M. Jagadeesh[a] and A. Varada Reddy[a]*

[a]*Analytical Division, Dept. of Chemistry, S.V. University, Tirupati-517502, India.*
[b]*Dept.of Biotechnology, SVIMS University, Tirupathi-517502, India*.

Abstract

Ni(II) and Co(II) complexes of benzyloxybenzaldehydethiosemicarbazone(L) were prepared and characterized by their physical, spectral and analytical data. The newly synthesized metal complexes have a composition of $[M(L)_2X_2]$, where M= Co(II) or Ni(II) and X= Cl. The electronic spectral studies show an octahedral geometry around Ni(II) and Co(II) complexes. The IR spectral data suggest the involvement of sulphur and azomethane nitrogen in coordination with the central metal ion. In order to evaluate the biological activity of the ligand and its metal, complexes have been screened for their antibacterial and antifungal activity against bacterial species like – *B.subtilis* and *S.aureus*(Gram-positive bacteria) and *E. coli, P. aeruginosa* and *P. vulgaris* (Gram-negative bacteria) and also fungal species like- *Microsporumgypseum, Teloschistes villous, Aspergillus fumigates and Candida albicans*. The results of antimicrobial studies clearly show that the process of chelation dominantly affects the overall biological behaviour of the compounds and they are potent against bacterial and fungal strains. The free ligand and its corresponding metal complexes were screened for antioxidant properties by radical scavenging methods such as 1,1-diphenyl -2-picryl hydrazyl (DPPH) and lipid peroxidation. They exhibited potent invitro - antioxidant activity dose dependently.

Keywords: Thiosemicarbazone, spectral; antioxidant activity, antimicrobial activity.

Introduction

Thiosemicarbazone ligands derived from the combination of a thiosemicarbazide and an aldehyde or ketone, are a useful ligand group for obtaining coordination spheres with mixed N/S donors. Interest in these ligands has been driven, in part, by potentially beneficial biological activity of ligands and their metal complexes, including, antifungal, antimicrobial, anticancer, fungicide, bacteriocide, anti-inflammatory and antiviral activities [1-6]. The inhibitory action of these compounds is attributed to their chelating properties [7-11].

In the present work, the syntheses and spectroscopic characterization of Ni(II) and Co(II) complexes of the ligand, benzyloxybenzaldehydethiosemicarbazone have been reported. The *invitro* antibacterial and antioxidant activities of these compounds were also extensively evaluated.

Antioxidants are widely studied for their capacity to protect organisms and cells from damage induced by oxidative stress during metabolism. Search for active components that prevent or reduce the impact of oxidative stress on cells is a contemporary field [12]. Exogenous chemicals in food system and endogenous metabolic processes in human body produce highly reactive free radicals, especially oxygen derived ones. Which are capable of oxidizing biomolecules and cause cell death and consequently cause tissue damage. Free radical oxidative processes also play a significant pathological role in causing human diseases. Many disease manifestations such as cancer, emphysema, cirrhosis, atherosclerosis and arthritis have been correlated with oxidative tissue damage. Also, excessive generation of reactive oxygen species (ROS) induced by various stimuli leads to variety of pathophysiological abnormalities such as inflammation, diabetes, genotoxicity and cancer [13]. In the present investigation, radical scavenging and antioxidative activity for the newly synthesized compounds are evaluated using two antioxidant methodologies.

Experimental Section

All the chemicals were commercially available and used as received. Ni(II) and Co(II) salts were used as chlorides. The melting point of the compound was obtained on a Sunbim (India) melting point apparatus. The IR spectra were recorded on a Nicolet FT-IR 560 Magna spectrometer using KBr (neat). The Bruker 300 MHz NMR spectrometer was used to obtain the ^{1}H NMR spectrum of the ligand. Elemental analyses were obtained from vario-micro qub elementar analyzer. The electronic spectra of complexes were recorded on Perkin Elmer UV/VIS Lambda 950. The metal content in the complexes were determined by using Fischer scope XDL-BZ, XRF instrument, Germany.

Synthesis of ligand (L)

Benzyloxybenzaldehydethiosemicarbazone was synthesized [14] by refluxing 2g of benzyloxybenzaldehyde in ethanol and 1g of thiosemicarbazide in ethanol mixture for about 1 hour. 30 min. in a round bottom flask. The light yellow coloured product obtained was separated by filtration and dried. The product was recrystalized from ethanol. The purity of the reagent was checked by TLC. Yield was found to be (65%): m.p 140-142^{0}C. ^{1}H NMR data ($CDCl_3$/TMS):

8.0-7.25 (m, 9H, Ar-H), 6.9 (d, 2H, NH_2), 11.25 (s, NH), 5.12 (s, N=CH), 3.18 (s, OCH_2). The elemental analysis data is as follows: Calc (%) for $C_{15}H_{15}N_3SO$: C, 63.13; H, 5.29; N, 14.73; S, 11.22. Found (%) C, 63.27; H, 5.37; N, 14.05; S, 11.09%.

Preparation of $[Ni(L)_2Cl_2]$ 1 and $[Co(L)_2Cl_2]$ 2 complexes

The complexes **1** and **2** were prepared by adding warm ethanolic solution of MX_2 (0.01M) [M= Ni or Co and X=Cl] to ethanolic solution of ligand (L) (0.02M) in about 30 mL of ethanol. The resulting solutions were refluxed for about 3 hours. The complexes thus formed were filtered and washed with water followed by ethanol. The resulting solid complexes were washed with water and dried. The physical and analytical data of ligand and its metal complexes were given in below

$[Ni(L)_2Cl_2]$ complex:

It is a light yellow coloured solid. It is decomposed at 270-273^{0}C. Yield was found to be 56%. IR(KBr,cm^{-1}) 3440 (NH_2),1616 (C=N), 810 (C=S), 696 (M-N), 400 (M-S). Anal.calcd for [$Ni(C_{15}H_{15}N_3SOCl)_2$]: C,51.45; H,4.31; N,12; S,9.1; Ni, 8.38 Found: C,51.62; H,4.33; N,11.89; S,9.18; Ni, 8.10.

$[Co(L)_2Cl_2]$ complex:

It is a light brown coloured solid. It is decomposed at >300^{0}C. Yield was found to be 47%. IR(KBr,cm^{-1}) 3337 (NH_2),1606 (C=N), 765 (C=S), 584 (M-N), 400 (M-S). Anal.calcd for [$Co(C_{15}H_{15}N_3SOCl)_2$]: C,51.41; H,4.32; N,12.2; S,9.15; Co, 8.41, Found: C,51.21; H,4.01; N,11.97; S,9.1; Co, 7.98.

Biological studies

Antimicrobial screening

The synthesized metal complexes and schiffs base ligand were screened for their antibacterial and antifungal activity against pathogenic bacterial species like – *E.coli, B.subtilis*, P.aeruginosa, Staphylococcus aureus and Proteus vulgaris and fungal species like- Microsporumgypseum, Teloschistes- villous, Aspergillus- fumigates and Candida albicans. The agar bioassay method [15] was adopted for the determination of antibacterial and antifungal activity as given under. Diluted inoculum (10^5 CFU/ml) of bacteria was spread on nutrient agar plates. In agar medium, the wells were punched and filled with the ligand and its corresponding metal complexes at three different concentrations (25, 50 and 100 µg/ml in DMSO solution were tested. DMSO was used as negative control and antibiotics such as ciprofloxacin and amphotrycin-B were used as positive control standards. The plates were incubated at 37 ^{0}C for 24 hours and then observed for the growth inhibition zones. The presences of clear zones around the wells have indicated that the compound is active towards bacteria. The diameter of zone of inhibition was calculated in millimetres with Himedia zone scale. The well diameter was deducted from the zone diameter to get the actual zone of the inhibition diameter.

***Invitro*-Antioxidants studies**

DPPH free radical scavenging activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical which has maximum optical absorbance at 517 nm. The reaction of DPPH with free radical scavenger causes decline in the

absorbance value at 517 nm [16-17]. The DMSO solutions of ligand(L) and its Cu(II) and Ni(II) complexes at 100 µM concentration were added to 100 µM DPPH in DMSO. The test tubes were kept at an ambient temperature for 20 minutes and the absorbances were measured at 517nm, against control. α-tocopherol was used as a positive control. These measurements were run in triplicate. The percentage of scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = [(A_{DPPH}-A_{TEST})/A_{DPPH}] \times 100$$

Where A_{DPPH} is the absorbance of DPPH without test sample (control) and A_{TEST} is the absorbance of DPPH in the presence of test sample.

Iron induced lipid peroxidation Assay

Male Albino rats (180-200 g) were used for the study. Prior to decapitation and removal of brain, the animals were anesthetized with ether and perfused transcardially with ice-cold normal saline to prevent contamination of brain tissue with blood. Tissue was weighed and its homogenate (10%w/v) was prepared in 150 mmol KCl and centrifuged at 800 rpm [18] for 10 minutes. The supernatant was used immediately for the study. Lipid peoxidation was induced by Fe^{+3}-ascorbate complex system in rat brain homogenate and estimated as thiobarbituric acid reacting substances (TBARS) by the method [19]. The incubation mixture contained in a final volume of 1.5ml, brain homogenate (500µl), KCl (150mmol) and DMSO (10 µl) or test compound dissolved in DMSO. Peroxidation was initiated by adding Fe^{+3} (100µM). After incubation for 20 minutes at 37 ^{0}C reactions were stopped by adding 2 ml of ice-cold 0.25M HCl containing 15% trichloro acetic acid (TCA), 0.38% thiobarbituric acid (TBA) and 0.05% butylated hydroxyl toluene (BHT). The samples were heated at 80 ^{0}C for 15 minutes, cooled and centrifuged at 1000 rpm for 10 minutes.The absorbance percentage inhibition of thiobarbituric acid reactive substances (TBARS) formed by test compounds were measured at 532 nm by using systronics UV/VIS spectrometer-117 instrument and calculated by comparing with the control. α-tocopherol was used as a positive control. The percentage inhibition of lipid peroxidation with selected ligand and its metal complexes and standard compounds were calculated using the following formula,

$$\text{Inhibition percentage} = [(A_{CONT} - A_{TEST})/A_{CONT}] \times 100$$

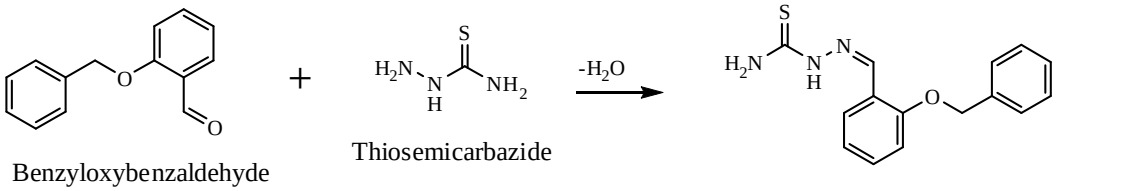
Where A_{CONT} is the absorbance of the control without test sample and A_{TEST} is the absorbance in the presence of the test sample.

Results and Discussion

The ligand (L) was synthesized by condensation of benzyloxybenzaldehyde with thiosemicarbazide in the presence of ethanol (Scheme-1).The complexes **1** and **2** were prepared by the reaction of the $NiCl_2.6H_2O$ and $CoCl_2.6H_2O$ with ligand (L) under the same conditions. The IR spectrum of the free ligand shows a band at 1586 cm^{-1} which is assigned to ν(C=N) stretching vibrational mode, which is a fundamental feature of azomethine group [20]. On complexation, these frequencies were observed to be shifted to higher wave number [21]. These observations suggest involvement of unsaturated nitrogen atoms of the azomethine groups in

bonding with the metal ion. The ν (C=S) band of the ligand is less intense in complexes and suggesting coordination of the metal through sulphur. Based on the elemental analysis and spectral studies, the proposed structures of the complexes formed were shown in fig (1).The bands within 700-400cm^{-1}and 600-300cm^{-1} are assigned to ν(M-N) and ν(M-S) stretching vibrations respectively. The electronic spectra of Co(II) complex showed two spin-allowed transitions at 17866 and 21744 cm^{-1} assignable to $^4T_1g(F)\rightarrow{}^4A_2g$, $^4T_1g(F)$ and $^4T_1g(F)\rightarrow{}^4T_1g(p)$ transitions respectively, are in conformity with octahedral arrangements for Co(II) ion. The appearance of a band at 19240 cm^{-1} due to $^3A_2g(F)\rightarrow{}^3T_1g$ (P) transitions favours an octahedral geometry [22], for the Ni(II) complex. The invitro antimicrobial properties of the ligand and their complexes were evaluated against Gram-Positive and Gram-negative bacteria and fugal strains. The antimicrobial results evidently show that the activity of ligand became more pronounced when coordinated to metal ions. The synthesized ligand and its metal complexes were screened for their antibacterial activity against *B.substills, S. aureus* (Gram-positive), *E.coli, P.aeruginosa*, and *P.vulgaris* (Gram-negative) bacteria and antifungal activity against *Teloschistes-villosus, Aspergillus-fumigates* and *Candida-albicans* at different concentrations. The standard drugs ciprofloxacin and amphotrycin were used as positive control and DMSO used as negative control. The results of antibacterial activity of ligand shows inactive against *B.substills, S.aureus* and *E.coli* and weak activity against *P.aeruginosa* and *p.vulgaris* when compared with standard ciprofloxacin. The Co(II) and Ni(II) shows more activity when compared to the standard drug ciprofloxacin. The zone of inhibitions of the antibacterial activity has been presented in (Table 1)

The antifungal activity results revealed that the ligand did not inhibit the growth of tested organism. The Co(II) and Ni(II) complex shows more activity when compared to the standard drug amphotrycin.(Table 2) represent the antifungal activity of ligand and its Co(II) and Ni(II) complexes.



Benzyloxybenzaldehyde Thiosemicarbazide Benzyloxybenzaldehydethiosemicarbazone

Scheme 1

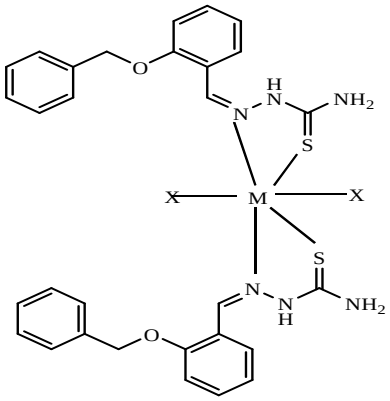


Fig.1 Proposed structure of benzyloxybenzaldehydethiosemicarbazone with Ni(II) or Co(II) complexes. Where M = Ni(II) or Co(II) and X= Cl

Table1.Antibacterial activity (Diameter of Zone of Inhibition mm) of ligand and its complexes (µg/ml)

Compound	**Micro-organism (Diameter of zone of inhibition in mm)**														
	B.substills			**S. aureus**			**E.coli**			**P.aeruginosa**			**P.vulgaris**		
	(Concentration µg/ml)														
	250	500	1000	250	500	1000	250	500	1000	250	500	1000	250	500	1000
Ligand(L)	-	-	-	-	-	-	-	-	-	06	09	15	08	11	13
1	16	20	27	08	13	21	07	11	19	07	15	17	-	-	-
2	06	10	19	09	12	21	07	13	20	12	18	25	-	-	-
Ciprofloxacin	18			16			18			16			18		

Table 2. Antifungal activity (Diameter of Zone of Inhibition mm) of ligand and its complexes (µg/ml)

Compound	**Micro-organism (Diameter of zone of inhibition in mm)**											
	Microsporum-gypseum			***Teloschistes-villosus***			***Aspergillus-fumigate***			***Candida-albicans***		
	(Concentration µg/ml)											
	250	500	1000	250	500	1000	250	500	1000	250	500	1000
Ligand(L)	-	-	-	-	-	14	-	-	-	-	-	-
1	10	12	19	09	11	20	10	11	18	10	12	16
2	06	10	18	10	12	21	07	09	10	08	10	17
Amphotrycin	13			13			14			14		

Table 3. Antioxidants scavenging activity of data of ligand (L) and its Ni (II) and Co (II) complexes at concentration of 100µM

Compound	DPPH scavenging (%)	Fe^{3+} induced lipid per oxidation (%)
Ligand(L)	46	38
1	2.7	31
2	23	21
α -tocopherol	53	65

The results of antimicrobial studies clearly show that the process of chelation dominantly affects the overall biological behaviour of the compounds, which are potent against bacterial and fungal strains.

The synthesized ligand and its metal complexes were screened for reduction of DPPH and inhibition of iron induced lipid peroxidation at 100 µM concentration. The antioxidants activity of ligand and its metal complexes in DPPH method as follows α –tocopherol > (L)> **2** > **1**, where as in lipid peroxidation the order of activity as follows α –tocopherol > (L)> **1** > **2.** Data have been presented in Table (5).

Conclusion

We have demonstrated a general route for preparation of Ni(II) and Co(II) complexes with Benzyloxybenzaldehydethiosemicarbazone and their structures are presented. The electronic spectra indicate that Ni(II) and Co(II) complexes have octahedral geometry. The results of antimicrobial studies clearly show that the process of chelation dominantly affects the overall biological behaviour of the complexes, which are potent against bacterial and fungal strains. The synthesized ligand and its metal complexes were screened for reduction of DPPH and inhibition of iron induced lipid peroxidation at 100 µM concentration. Based on the results obtained, the free ligand was found to be good antioxidant, as comparable with standard α-tocopherol. However, its antioxidant activity can be further confirmed by *invivo* methods.

Acknowledgements

The authors are thanking to CSIR, New Delhi, Govt. of India, for financial assistance provided to Dr. S.Lakshminarayana as a form of SRF.

References

[1] R Tudor; G Aurelian; N Anca ; G Rodica. *Molecules*., **2007**, 12(4), 782-796.
[2] C Suresh ;T Monika. *J.Serb.Chem.Soc*., **2008** 73, 727-734.
[3] CK Noriko; S Kiyoshi; K Chisa; S Nobuhiro; I Motoki; N Kenji. *J. Inorg. Biochem*., **2001** 84(1-2) 55–65.
[4] DX West; SB Padhye; PB Sonawane. Struct. *Bonding*., **1991** 76(4) 11-22.
[5] Z Afrasiabi; E Sinn; S Padhye. *J. Inorg. Biochem*., **2003**, 95(4) 306–314.
[6] EM Jouad; G Larcher; M Allain. *J. Inorg. Biochem*., **2001**, 86(2-3) 565–571.

[7] NK Singh; SB Singh . *Indian J. Chemistry*., **2001**, 40(10) 1070–1075.
[8] Z Afrasiabi; E Sinn; *J Chen* . *Inorg. Chim. Acta.*, **2004**, 357(1), 271–278.
[9] E Labisbal; KD Haslow; A Sousa-Pedrares; J Valdes- Mart´ınez; S Hernandez-Ortega; DX *West. polyhedron.*, **2003**, 22(20), 2831–2837.
[10] DK Ajay kumar; AP Sangamesh ; SB Prema. *Int. J. Electrochem. Sci*.., **2009**, 4, 717-729.
[11] RV Singh; N Fahmi; MK Biyala. *J. Iran. Chem. Soc*., **2005**, 2(1), 40-46.
[12] HH Hussain; G Babic; T Durst; J Wright; M Flueraru; A Chichirau; LL Chepelev. *J. Org. Chem.,* **2003**, 68, 7023—7032.
[13] J Mc Clements; Decker. J. Food. Sci., **2000**, 65(8) 1270—1282.
[14] S Lakshmi Narayana; K Janardhan Reddy; S Adinarayana Reddy; Y Sarala ; A Varada Reddy . *Food .Anal. Methods*., **2008**, 1(4) 293–299.
[15] C Sheikh; MS Hossain; MS Easmin; MS Islam; M Rashid. *Biological & Pharm. Bull*., **2004**, 27, 710 -713.
[16] T Hatano; H Kagawa; T Yasuhara ; T Okuda . *Chem. Pharm. Bull*., **1988**, *36, 2090-2097.*
[17] MS Blois. *Nature*., 1**958**, 181, 1199-1200.
[18] PK Chaudhuri; R Srivastava; S Kumar. *Mol. Cell.Biochem*., **2000**, 211, 69-77.
[19] K Bharathi; G Swarna Latha; SK Arifa Begum; KVSRG Prasad. *J. Pharm. Res*., **2008**, 7, 79-82.
[20] LJ Boucher; WV Day. Electrochemical Method, 2nd Ed., Willey New York., **1977**, 6-13.
[21] EL Mustapha Jouad; A Magalia; AK Mustayeen ; MB Gilles. *Polyhedron.*, **2005**, 24, 327-332.
[22] ANM Kasim ; D Venkappayya ; GV Prabhu. *J. Indian Chem. Soc*., **1999**, 76, 67-69.