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Article

Synthesis, Spectral Characterization and Antioxidant Studies of Ruthenium(II) Hydrazone Complexes

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ABSTRACT

New ruthenium(II) complexes with hydrazone ligand and 4-Bromo benzoic acid (2-hydroxy-1-naphthylidene) hydrazide **(1)** were synthesized. Structural features of the ligand and complexes were determined by various physicochemical and spectral techniques. The hydrazone ligand acts as tridendate ligand with ONO as the donor sites and is preferably found in the enol form in the complexes. The radical scavenging ability, assessed using a series of antioxidant assays involving 2,2-diphenyl-2-picrylhydrazyl radical, hydroxyl radical, and nitric oxide radical, showed that the complexes possess significant radical scavenging activity.

Key words: Hydrazone, Ru(II)complexes, Spectral studies, Antioxidant

1. INTRODUCTION

Inorganic biochemistry is an emerging field of science that utilizes transition metal complexes for various application in biological, medical, and environmental sciences [1,2]. Transition metal complexes have been utilized for the design and development of metal-based chemotherapeutic agents that are capable of binding and cleaving nucleic acid under physiological conditions [3,4]. To date, metal-based compound cisplatin is one of the most widely used anticancer drug. Although 70% of all cancer patients receive cisplatin during cancer treatment, chemotherapy with cisplatin and its analogues still has several drawbacks; toxic side-effects and lack of activity (drug resistance) against several types of cancer are problems which need to be overcome [5-8]. These limitations have aroused interest toward the design and evaluation of transition metal complexes other than platinum derivatives for therapeutic use. At this juncture, ruthenium metal has emerged as an attractive alternative for the platinum group due to several favorable properties suited to rational anticancer drug design and biological applications. The entrance of two ruthenium-based drugs, NAMI-A and KP109 into clinical trials for the treatment of metastatic tumors increased the interest in this metal [9-11]. Both complexes behave quite differently from cisplatin *in vivo*. In addition, a number of Ru(II) compounds were recently shown to possess very encouraging cytotoxic and antitumor properties in pre-clinical models and are now under active investigation [12-14]. Over the years, much attention has been paid to investigate the chemistry of transition metal complexes with large number of hydrazones as coligand. Hydrazone ligand is promising compounds from the view point of coordination chemistry because of their ability toward complexation and wide range of biological and non-biological properties. The chemistry of transition metals with ligand from the hydrazone family has been of interest to coordination as well as bio-inorganic chemists due to their different bonding modes with both electron-rich and electron-poor metals [15-17]. This aroused our interest in the design of ruthenium(II) salicylaldehyde hydrazone complexes with a view point toward evaluating anticancer properties. Herein, we report the synthesis, characterization of ruthenium(II) complexes containing hydrazone ligand derived from condensing 4-bromo benzoic acid hydrazide with 2-hydroxy-1-naphthaldehyde. The investigation of the biological properties of the ligand and ruthenium(II) complexes has been focused on the antioxidant properties against 2,2-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl (OH), and nitric oxide (NO) radicals.

2. EXPERIMENTAL DETAILS

2.1. Materials and Instrumentation

All the chemicals used were of chemically pure and AR grade. Solvents were purified and dried according to the standard procedure [18]. The metal precursors $[RuHCl(CO)(PPh_3)_3]$ $[RuHCl(CO)(AsPh_3)_3]$ were prepared by literature methods [19]. Melting points were recorded with Veego VMP-DS heating table and elemental analysis (C, H, and N) were performed on a Perkin Elmer 240C elemental analyzer at University of Hyderabad, Hyderabad, India. Infrared spectra were recorded as KBr pellets method in the range of 400–4000 cm^{-1} using Perkin Elmer FT-IR 8000 spectrophotometer. Electronic spectra were recorded in DMSO solution with a systronics double beam UV-Vis spectrophotometer 2202 in the range of 200–800 nm. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV III 500 MHZ instrument using TMS as an internal reference. ^{31}P NMR spectra were recorded on a Bruker AV III 500 MHZ instrument using orthophosphoric acid as an internal reference. Antioxidant studies were carried out at the Kovai Medical Centre and Hospital Pharmacy College, Coimbatore, Tamil Nadu.

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2.2. Preparation of Hydrazone Ligand

A methanolic solution (50 mL) of 4-bromo benzoic acid hydrazide (0.01 mol) was added to a methanolic solution (25 mL) containing 2-hydroxy-1-naphthaldehyde (0.01 mol). The mixture was refluxed for 1 h which results in the formation of a precipitate. The reaction mixture was cooled to room temperature and the solid compound was filtered, washed, and recrystallized from ethanol [Scheme 1].

2.2.1. 4-bromo benzoic acid (2-hydroxy-1-naphthylidene) hydrazide (1)

Color: Yellow; Yield: 89%; M.P: 296°C. Anal. calcd. for $C_{18}H_{13}BrN_2O_2$ (%): C, 58.56; H, 3.55; N, 7.59 Found (%): C, 58.51; H, 3.58; N, 7.64. IR (KBr, cm^{-1}): 1602 ν(C=N); 1641 ν(C=O); 3176 ν(NH); 3437 ν(Ph-OH). UV–vis (DMSO), λmax (nm): 263, 310 (π→π*, n→π*). ^{1}H NMR (DMSO-d_6); δ 9.97 (s, 1H,-NH); δ 8.50 (s, 1H, H-C=N); δ 11.33 (s, 1H, Ph-OH); δ 6.31–7.94 (m, 10H, aromatic).^{13}C NMR (DMSO-d_6); δ 162 (C=O); δ 149 (C=N); δ 102–139 (aromatic).

2.3. Preparation of Ruthenium(II) Complexes

4-bromo benzoic acid (2-hydroxy-1-naphthylidene) hydrazide **(1)** was added to a solution of $[RuHCl(CO)(EPh_3)_3]$ (E = P/As) in 1:1 molar ratio in ethanol-benzene and the reaction mixture was refluxed for 6 h. The progress of the reaction was monitored using TLC. At the end of the reaction, the solution was concentrated to about 3 mL and petroleum ether (60–80°C) was added whereby the solid separated out. The obtained solid was recrystallized from CH_2Cl_2/petroleum ether and dried under vacuum [Scheme 2].

Where, E= P/As

2.3.1. $[Ru(CO)(PPh_3)_2L]$ (2)

Color: Orange; Yield: 84%; M.P: 232–237°C. Anal. calcd. for $C_{55}H_{41}BrN_2O_3P_2Ru$ (%): C, 64.71; H, 4.05; N, 2.74. Found (%): C, 64.75; H, 4.09; N, 2.77; IR (KBr, cm^{-1}): 1594 ν(C=N); 1343 ν(C-O); 1952 ν(C≡O) cm^{-1}. UV-vis (DMSO), λmax (nm): 266, 305 (ILCT), 420 (MLCT). ^{1}H NMR (DMSO-d_6); δ 9.02 (s, 1H, H-C=N); δ 6.45–7.95 (m, 40H, aromatic).^{13}C NMR (DMSO-d_6); δ 156, 157 (C-O); δ 152 (C=N); δ 193 (C≡O); δ 120–138 (aromatic).^{31}P NMR (DMSO-d_6); δ 37.12.

2.3.2. $[Ru(CO)(AsPh_3)_2L]$ (3)

Color: Brown; Yield: 84%; M.P: 229–235°C. Anal. calcd. for $C_{55}H_{41}BrN_2O_3As_2Ru$ (%): C, 59.58; H, 3.73; N, 2.53. Found (%): C, 59.63; H, 3.76; N, 2.59; IR (KBr, cm^{-1}): 1590 ν(C=N); 1341 ν(C-O); 1960 ν(C≡O) cm^{-1}. UV-vis (DMSO), λmax (nm): 265, 319 (ILCT), 410 (MLCT). ^{1}H NMR (DMSO-d_6); δ 9.32 (s, 1H, H-C=N); δ 6.66–7.97 (m, 40H, aromatic).^{13}C NMR (DMSO-d_6); δ 156, 158 (C-O); δ 153 (C=N); δ 197 (C≡O); δ 120–140 (aromatic).

2.4. Antioxidant Assays

The DPPH radical-scavenging activity of the compounds was measured according to the method of Elizabeth [20]. The DPPH radical is a stable free radical having a λ_{max} at 517 nm. A fixed concentration of the experimental compound (100 µL) was added to a solution of DPPH in methanol (0.3 mM, 1 mL) and the final volume was made up to 4 mL with double distilled water. DPPH solution with methanol was used as a positive control and methanol alone acted as a blank. The solution was incubated at 37°C for 30 min in dark. The decrease in absorbance of DPPH was measured at 517 nm.

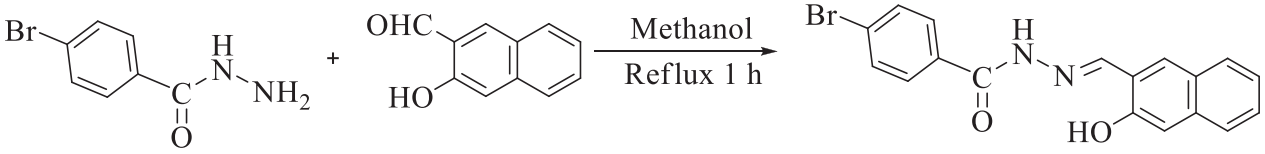
The scavenging activity for OH radical recommended by Yu *et al*., with major changes [21]. Reaction mixture contained 0.6 mL of 1.0 mM Deoxy ribose, 0.4 mL of 0.2 mM phenyl hydrazine, and 0.6 mL of 10 mM phosphate buffer (pH 7.4). It was incubated for 1 h at room temperature. Then, 1 mL of 2–8% trichloro acetic acid, 1 mL of 1% thiobarbituric acid, and 0.4 mL of extract at various concentrations were added and kept in water bath for 20 min. The absorbance of the mixture at 532 nm was measured with a spectrophotometer. The OH radical-scavenging activity was calculated.

NO radical scavenging activity was determined based on the procedure by Griess Illosvoy reaction [22]. The reaction mixture (3 mL) containing sodium nitroprusside (10 mM, 2 mL) and phosphate buffer saline (0.5 mL) at different concentration and standards (50–250 µg mL^{-1}) were incubated at 25°C for 150 min. After incubation, 0.5 mL of the incubated solution containing nitrite was pipetted and mixed with 1 mL of sulfanilic acid reagent and allowed to stand for 5 min for completing diazotization. Then, 1 mL of N-1-naphthyl ethylene diamine dihydrochloride was added, mixed, and allowed to stand for 30 min at 25°C. The absorbance of pink colored chromophore formed during diazotization was measured at 540 nm.

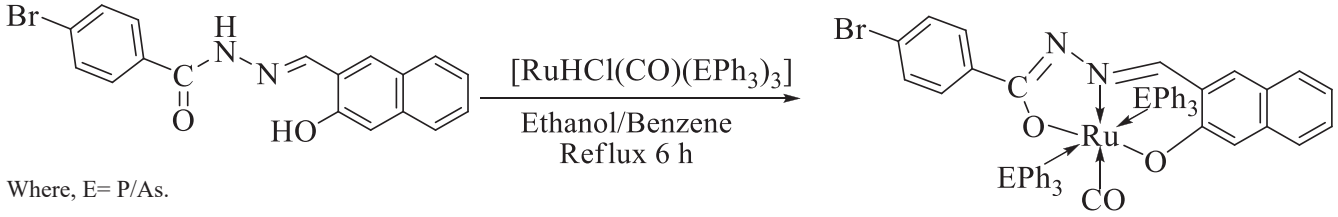
For each of the above assays, the tests were run in triplicate by varying the concentration. The percentage inhibition of absorbance was calculated and plotted as a function of the concentration of standard and sample to determine the antioxidant concentration. The percentage activity was calculated using the formula % activity = ([Ao–Ac]/Ao) × 100, where Ao and Ac represent the absorbance in the absence and presence of the test compounds, respectively. The 50% of activity (IC_{50}) is calculated from the result of percentage activity. Ascorbic acid (Aca) was used as a standard for all the above assays.

3. RESULTS AND DISCUSSION

Analytical and spectroscopic data for the ligand and ruthenium complexes indicate a 1:1 metal-ligand stoichiometry for all the



Scheme 1: Synthetic route of the hydrazone ligand.



Where, E= P/As.

Scheme 2: Synthetic route of the ruthenium(II) hydrazone complexes.

complexes. The synthetic route of the ligand and the proposed structure of the complexes are shown in Schemes 1 and 2.

3.1. Infrared Spectroscopy

The important IR spectra of the ruthenium(II) hydrazone complexes were compared with that of the respective free hydrazone ligand in the region 4000–400 cm^{-1}. The spectra of the ligand displayed a medium sharp band at 1602 cm^{-1} which can be assigned to the azomethine C=N stretching frequencies. In the spectra of the complexes, these bands shift toward lower wave number at 1594 and 1590 cm^{-1} reveals that the coordination of the azomethine nitrogen to the metal center. A band appears at 1641 cm^{-1} for ligand due to vibration of the C=O which disappears in the spectra of the complexes and a new band for C–O appears at 1343 and 1341 cm^{-1} indicating that the other coordination through keto oxygen after enolization followed by deprotonation on oxygen. The medium intensity bands which appears at 3437 cm^{-1} corresponding to phenolate OH of the free hydrazone ligand. On complexation, these bands completely disappear. This observation clearly indicates the coordination of phenolic oxygen to the ruthenium center. In all the complexes, the strong band in the region 1952 and 1960 cm^{-1} was due to terminally coordinated ruthenium carbonyl group. Overall, the complexes contain dibasic ONO coordinated hydrazones. In addition, the characteristic absorption bands due to triphenylphosphine and triphenylphosphine complexes were also observed in their expected regions [23].

3.2. Electronic Spectra

The electronic spectra of the ligand exhibited bands at 263 and 310 nm. The first band at 263 nm is attributed to π→π* transition, while the band at 310 nm is assigned to n→π* transition associated with the imine and keto function of the hydrazone. The spectra of the complexes observed three bands in the region 265–420 nm. The bands at 265–266 and 305–319 nm are characterized as ligand centered transitions occurring within the ligand orbitals. These bands associated with the intra ligand transitions. These bands are shifted when compared to ligand indicating the involvement of imine nitrogen and keto oxygen in coordination with ruthenium ion. The band appearing in the region 410–420 nm is assigned to charge transfer transitions arising from the excitation of an electron from metal t_{2g} level to an unfilled molecular orbital derived from the π*level of the ligand. The pattern of the electronic spectra of the complexes indicated the presence of an octahedral environment around the ruthenium ion, similar to that of other ruthenium(II) octahedral complexes [24].

3.3. NMR Spectra

The ^{1}H NMR spectra of the free ligand show a singlet in the region δ 9.97 for the hydrazine NH protons, which is absent in the spectra of the complexes indicating the enolization and deprotonation of the –NH–C=O group before coordination of ligand to metal through keto oxygen. In addition, the ligand shows a sharp singlet for azomethine (HC=N) proton at δ 8.50. However, in the case of ruthenium complexes, the signal corresponding to the azomethine gets shifted slightly downfield due to participation of azomethine nitrogen in coordination with the metal ion and observed at δ 9.02 and 9.32. The proton of the phenolic OH group appears as a singlet at δ 11.33 for the free ligand. In the spectra of the complexes, the resonance arising from the OH proton is not observed, indicating the coordination of the OH oxygen to the metal ion. The aromatic protons of the ligand exhibited a multiplet in the region δ 6.31–7.94, on complexation the protons on the phenyl ring, remain more or less unchanged in the complexes in the region δ 6.45–7.97 due to the delocalization of electron density in the system and these signals in the complexes cannot be distinguished from the aromatic signals due to their extensive overlap [25].

The ^{13}C NMR spectra of the ligand displayed well-defined signal at δ 162 corresponds to the carbonyl carbon (C=O), which disappears in the spectra of the complexes and appears as a new signal at δ 157 and 158 (C–O) indicating the coordination of oxygen *through* deprotonation. The spectra of the ligand show a single resonance at δ 149 due to the azomethine carbon atom, in the complexes show a signal at δ 152 and 153 clearly indicates the coordination of C=N. The aromatic carbons of the free ligand and complexes show signal in the region δ 102–140. For all the complexes, the terminal carbonyl group C≡O appears at δ 193 and 197 [26].

To confirm the presence of triphenylphosphine group and to determine the geometry of the complex,^{31}P NMR spectra were recorded. A sharp singlet was observed at δ 37.12 for the complex **2** due to presence of magnetically equivalent phosphorous atoms suggesting the presence of two triphenylphosphine groups in a position trans to each other [27].

3.4. Antioxidant Activities

It is well known in the literature that transition metal hydrazone complexes displayed significant antioxidant activity [28]. Hence, we investigated the antioxidant potential of the newly synthesized ruthenium(II) hydrazone complexes against DPPH, OH, and NO radical scavenging assays.

3.4.1. OH radical

OH radical is a highly oxygen-centered radical formed from the reactions of various hydroperoxides with transition metal atoms. Among all the free radicals, OH radical is by far the most potent and therefore the most dangerous oxygen metabolic and hence the elimination of this radical is one of the major aims of antioxidant administration [29]. It attacks proteins, DNA, and polyunsaturated fatty acid in membranes and most biological molecules [30]. OH radical is known to be capable of abstracting hydrogen atoms from membrane lipids and brings about peroxide reaction of lipids. Scavenging activity of the ruthenium complexes on OH radical has been investigated and compared with the standard Aca. The IC_{50} values indicated that the compounds showed antioxidant activity in the order of 2>3>1. It reveals that Complex 2 showed significant antioxidant activity compared to Aca. The comparison of antioxidant activities of tested compounds along with the standard are given in Figure 1.

3.4.2. DPPH radical

The DPPH assay is widely used for assessing the ability of radical scavenging activity and it is measured in terms of IC_{50} values. Because of the presence of odd electron, DPPH shows a strong absorption band at 517 nm in the visible spectrum [31]. As this electron becomes

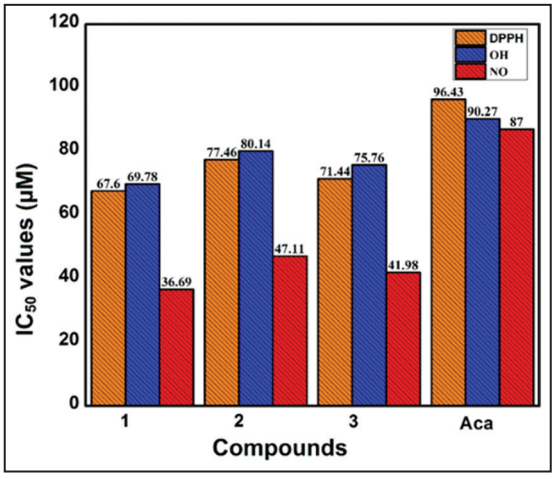


Figure 1: Comparison of IC50 values for antioxidant activities

Table 1: Antioxidant activity of the compounds

Compounds	IC_{50} µM		
	DPPH	OH	NO
1	67.60	69.78	36.69
2	77.46	80.14	47.11
3	71.44	75.76	41.98
Ascorbic acid (Aca)	96.43	90.27	87.00

paired off in the presence of a free radical scavenger, this absorption vanishes, and the resulting decolorization is stoichiometric with respect to the number of electrons taken up. The DPPH assay of the tested compounds is shown in Figure 1, it is seen from the results that, all the complexes exhibited moderate activity compared to the Aca. The IC_{50} values indicated that the compounds showed antioxidant activity in the order of 2>3>1. Complex 2 showed a higher antioxidant activity compared to other complexes.

3.4.3. NO radical

NO is a diffusible free radical which plays a role as an effectors in diverse biological systems including neuronal messenger, vasodilatation, and antimicrobial and antitumor activities. NO inhibitors were shown to have beneficial effects on some aspect of inflammation and tissue damage seen in inflammatory diseases [32]. These compounds are responsible for altering both the structural and functional behavior of many cellular components. The metal hydrazones have the property to counteract the effect of NO formation and, in turn, possess considerable interest in preventing the ill effects of excessive NO generation in the human body. To understand the ability of the ruthenium complexes toward the reactive radical species, a NO scavenging assay of them was carried out and the results are presented in Figure 1. It is seen from the results that, ruthenium complexes exhibited higher antioxidant activities than that of the Aca. The IC_{50} values indicated that the compounds showed antioxidant activity in the order of 2>3>1. Complex 2 showed higher scavenging activity than the rest of the complexes.

In general, the antioxidant activity of the ruthenium(II) hydrazone complexes against the three different radicals, that is, DPPH, OH, and NO confirmed that the complexes showed greater antioxidant activity and obtained results clearly indicated that the complexes are more effective to arrest the formation of OH than the DPPH and NO radicals [Table 1]. The observed lower IC_{50} values in antioxidant assays did demonstrate that the complexes have the potential as drugs to eliminate the radicals.

4. CONCLUSION

New ruthenium(II) complexes bearing ONO hydrazone ligand and the PPh_3/$AsPh_3$ as coligand are synthesized and characterized by various spectroscopic techniques. Based on the spectroscopic studies, an octahedral geometry has been tentatively assigned for the Complexes 2 and 3. The antioxidant activity revealed that complexes can serve as potential antioxidant against OH radical than DPPH and NO radicals. In particular, the Complex 2 shows excellent activity against OH radical. Results obtained from our presented work would be useful to understand the mechanism of interactions of the compounds with free radical and helpful in the development of their potential biological, pharmaceutical, and physiological implications in the future.

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***Bibliographical Sketch**

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