

ORIGINAL RESEARCH ARTICLE

Synthesis, Characterization and Nuclease Activity of some Novel Palladium (II) - Complexes of Benzil Thiosemicarbazone (BTSC) and Substituted Thiosemicarbazones

M.Adharvanachary*¹, Prakash.MMS.Kinthada*² and P.Kalyani³

¹Department Of Complexity Science and Engineering, Division Of Trans Disciplinary Science, University Of Tokyo, JAPAN

²Department of Chemistry, GIT, GITAM University, Rushikonda, Visakhapatnam, 530045.INDIA

³Department Of Chemistry, Indira priyadarshini Institute of Technology, Jawaharlal University of Technology, Hyderabad, INDIA

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ABSTRACT

Some Hitherto unknown complexes of Benzil Thiosemicarbazone and Substituted Thiosemicarbazones with Au(III) have been synthesized and characterized by elemental Analysis, spectral viz. FT-IR, NMR, UV-Vis, Mass etc, magnetic, thermal and conductance studies. All complexes are crystalline powders decomposed by mineral acids. The spectral and other data indicate that all the Pd (II) complexes are Square Planar. The ligand, its Schiff's Bases and metal chelates would be screened *in vitro* for anticancer activity against some cancer Cell lines.

Key words: Benzil Thiosemicarbazone, FT-IR, NMR, UV-Vis Mass, Metal chelates.

INTRODUCTION

Since Rosenberg's discovery of cisplatin cytotoxic activity in the 1960s the preparation of metallocomplexes with potential antitumor activity has been one of the main challenges of transition metal chemistry. As a consequence, thousands of cisplatin analogs have been synthesized by varying the nature of the leaving groups and the carrier ligands [1-4]. Nowadays, not only platinum-containing complexes are extensively studied with the aim of broadening the spectrum of transition metal-based complexes that could be used in the treatment of cancer. Mainly due to the similar structural properties, palladium (II) complexes were tested on various cancer cells among the first of the non-platinum metallocomplexes [5,6]. In addition, platinum and palladium complexes, in particular organometallic derivatives, have applications in catalytic and synthetic processes [7,8], as chiral auxiliaries [9] or as building blocks for molecular architectures of higher complexity [10]. They also show interesting mesogenic [11, 12] and luminescent and electronic properties [13,14]. Thiosemicarbazone ligands (TSCs) possess antiviral, antimalarial, antifungal, antimicrobial and antitumor activity and they are

among the most potent inhibitors of the ribonucleotide reductase activity [15-22]. These activities are normally increased upon coordination, so they are suitable ligands for the synthesis of potential anticancer agents.

EXPERIMENTAL

Chemicals and reagents:

All the reagents used in the preparation of ligands and their metal complexes were of reagent grade (Merck). The solvents used for the synthesis of ligands and metal complexes were distilled before use. All other chemicals were of AR grade and used without further purification.

The elemental analysis was performed by using micro analytical techniques. The IR spectra were recorded in the range 4000-200cm⁻¹ using KBr discs with Perkin-Elmer model 1430 and 337. The electrical conductivity measurements were made in DMF (10⁻³ M) at room temperature (27 ± 2^oc) using a Digisun digital conductivity meter (DI-909 model). The NMR spectra were recorded in DMSO-d₆ on NMR spectrophotometer model JEOL Ex-90 FT using TMS as the reference. The magnetic susceptibilities were determined at Room temperature, on a Guoy balance using

Mercury Tetrathiocyanato Cobalt (II) as a magnetic standard. Molecular weights of the complexes were determined by cryoseopic method using camphor as solvent. Magnetic measurements were carried, out in the polycrystalline state on a PAR model ISSfifl vibrating sample magnetometer operating at field strength of 2-1.0 KG. High purity nickel metal (saturation moment 55 emu/g) was used as a standard.

Synthesis of Ligands:

A 1x10⁻³M solution prepared by dissolving appropriate amounts of thiosemicarbazide and substituted thiosemicarbazides in 50 ml methanol and 2ml of glacial Acetic acid was added dropwise to a 5X10⁻²M solution of Benzil in 50 ml Methanol while stirring and refluxed for 2-3 hours and the product that separated was recrystallized in Methanol. Identification of the product was based on Elemental analysis, Ft-IR, NMR, Uv-Vis, Mass data as described earlier.

Synthesis of metal complexes:

To 30ml of Pd(II) solution (5x10⁻²M) in Methanol was added (1x10⁻²M) BTsc solution in methanol and the mixture refluxed for about 1 hour. The solid that separated was filtered and washed with water and recrystallized with methanol.

Plasmid isolation:

The E. coli DH5alpha strains containing plasmid pBR 322 was grown in Luria broth (LB) medium supplemented with 100 Micro g/ml ampicillin. Cells from 5 ml culture were harvested by centrifuging the culture for 10 minutes at 8000 rpm. Plasmid pBR 322 was isolated using Qiagen column following manufacturer's protocols.

Assay Of Nuclease activity:

The DMF solution containing metal complexes was taken in a clean eppendroff tube and 1 micro gram of plasmid DNA was added. The contents were incubated for 30 min at 37°C and loaded on 0.8% agarose gel after mixing 5 micro literes of loading buffer (0.25% bromophenol blue + 0.25% xylene cyanol + 30% glycerol sterilized distilled water). Electrophoresis was performed at constant voltage till the bromophenol blue reached to the 3/4 of the gel. Further the gel is stained for 10 min by immersing it in Ethidium bromide solution (5 micro g/ml of H₂O). The gel was then de-stained for 10 min by keeping it in sterile distilled water and plasmid bands were visualized by viewing the gel under transilluminator and photographed [27].

RESULTS AND DISCUSSION

Infra Red Spectra of the ligand:

Identification of the synthesized alloxane thiosemicarbazones was carried out by taking the results of its elemental analysis and the IR and NMR spectra into consideration. The Infra Red Spectra measured in KBR pellets showed absorption peaks that were assigned to the stretching vibrations of an azomethine bond (=C=N-) at 1610 and 1535 cm⁻¹. The peaks at 1075, 1020, 840 and 745 cm⁻¹ are assigned to the C=S bond and a band corresponding to -NH is observed at 3325 cm⁻¹.

Infra Red Spectra of Complexes:

The important vibrational bands of metal complexes have been obtained. The absence of SH band at 2570 cm⁻¹ and presence of NH band at 3328 cm⁻¹ in the IR spectrum of ligands suggest that the ligands remain in thione form at least in solid state. The IR spectra suggests that in all complexes coordination occurs through the azomethine nitrogen and thioketo sulphur atom. The band at 1610 is lowered indicating the coordination of the azomethine Nitrogen atom [19]. The bands at 1070, 1020, 830 and 740 cm⁻¹ assigned to the v(c=s) band are shifted towards a lower frequency indicating the sulphur coordination [20]. Additional bands are observed in Far IR spectra of metal complexes in 460-410 and 270-260 cm⁻¹ regions due to v (M-N) and v (M-S) modes respectively. Based on molecular weight determination, magnetic moments, electronic and IR spectra a general structure and square planar geometry is assigned for the Benzil thiosemicarbazone and substituted thiosemicarbazone complexes.

¹H NMR Spectra:

A peak at 9.2-9.8 ppm which is attributed to the NH group indicates that the ligands are in thione form. The NMR spectrum of ligands shows different signals 8.4 and 7.24 ppm aromatic proton, δ7.60 ppm (NH₂ protons) of thiosemicarbazide moiety [13]. These signals are not changed in the NMR spectra of the metal complexes. The H-NMR spectrum of BTsc was recorded in 4-DMSO solvent. It shows signals corresponding to -NH₂, NH (hydrazone) and -OH protons at 2.18 (s, 3H), 7, 22-7.52 (m, 5H), 8.76-8.14 (2H), 10.68 (s, 1H) and 11.74 (s, 1H) respectively. The nmr spectrum of metal chelates confirms the non participation of NH₂ group in the coordination with metal ions. ¹H NMR signals are well defined and the spectrum of free ligand [28]

exhibits two resonances for the NH₂ protons at 7.8 ppm, a result explained in terms of hindered rotation about the C(S)-4NH₂ bond due to its partial double bond character [29,30]. The metal complexes show only one resonance due to 4NH₂ protons, upfield for some complexes (at 9.46, 8.30, 8.23) and down field (at 9.72) for some complexes. The signal at 11.90 ppm in the spectrum of ligand due to 2'NH is present in most of the complexes with down field shifts (11.40, 11.43, 11.63) and with an upfield shift for one complex (12.41) probably indicating a change in the nature of NH resonance.

¹³C NMR:

¹³C-NMR spectral data indicate in all the complexes downfield or upfield chemical shifts are obtained for the carbon resonances adjacent to the assumed coordination sites while the others remain essentially unchanged. The affected carbon resonances in the ligands are shifted or absent, supports coordination of azomethine nitrogen to metal.

All the metal complexes are stable at room temperature non hygroscopic, sparingly soluble in methanol or ethanol and fairly soluble in DMF and DMSO. The analytical data for ligand and metal chelates are consistent with their proposed molecular formulae. The molar conductivity data (Table 1) of the Pd (II) thiosemicarbazone and Substituted

BTSC indicates that all the metal complexes are non electrolytes and are monomers. The presence of chloride is evident only after the chemical decomposition of metal complexes suggesting the presence of chloride in the complex. The magnetic moment values are observed as zero for complexes.

Mass Spectra:

The mass spectrum of ligand and metal complexes is recorded under liquid secondary ion mass spectral conditions [31-33]. The ligands all Tsc and Sust. Tscs gave the peaks at m/z 166 and 178 Da₁ and these values confirm the molecular weight of the ligands. The LSIM spectra of the complexes of All.Tsc and Substituted TSCs showed abundant

ions at m/z 190 and 570, 578, 664, 846. The ions support the proposed composition and structure.

Electrons spin resonance spectra:

Electronic spin Resonance spectra of Palladium complex was recorded in DMF at liquid nitrogen temperature value equal to 24.

Electronic Spectra:

In square planar complexes of Pd(II), two energy level sequences [34,35] viz. $d_{z^2} < d_{xz} < d_{y^2} < d_{xy} < d_{x^2-y^2}$ and $d_{xz} < d_{yz} < d_{z^2} < d_{xy}$, $d_{x^2-y^2}$ have been suggested. The former has recently been studied by X-Ray and polarized single crystal spectral studies [24] of the Palladium Ions. Three spin allowed d-d transitions from the three lower lying d levels to the empty $d_{x^2-y^2}$ orbitals are predicted on the basis of this sequence. Accordingly the bands observed in the regions 16,100-23,800; 23,800-28,900; 28,900-35,400 cm⁻¹ may be assigned to the transitions 1A_{1g}-1A_{2g}, 1B_{1g}-1B_{1g} and 1A_{1g}-1E_{1g} respectively. The peaks observed for the Palladium(II) complexes at 22,240-222,260, 27800-28200 and 37,046-37,200 cm⁻¹ support the square planar geometry.

Molar Conductance studies:

The molar conductivity data suggest that the complexes are non electrolytes.

Magnetic Studies:

The magnetic moments (Table 1) of metal complexes are found to be subnormal which may be attributed to the presence of magnetically coupled metal centers in dimeric complexes.

Thermal Analysis:

In the present investigations on the thermal behavior of the Palladium (II)-Benzil Thiosemicarbazone and Substituted Thiosemicarbazones do not have any water molecules.

The Benzil Thiosemicarbazone and substituted thiosemicarbazone and their metal complexes are stable at room temperature, non hygroscopic, insoluble in H₂O, but slightly soluble in ethanol and methanol and readily soluble in DMF and DMSO. The colour, molecular weight and molar conductance data are summarized in Table.1:

Table 1: Physical and analytical data of the Pd (II) complexes of Benzil Tsc and Substituted Tscs

Ligand/Complexes	Colour	Yield	Mol.Wt	Conductance Ohm-1.cm2.mol-	M.P0
[Pd(II)BTsc]	Brown	98%	389.77	28	204
[Pd(II)B.Me Tsc]	Brown	86%	404.78	24	216
[Pd(II)B. EtTsc]	Brown	94%	418.76	21	200
[Pd(II)B.Ip Tsc]	Red	92%	432.78	26	198
[Pd(II)B.Cy Tsc]	Brown	98%	462.74	29	226
[Pd(II)B.Ph Tsc]	Red	82%	466.78	20	220
[Pd(II)Benz Tsc]	Brown	88%	480.02	21	236
[Pd(II)Otol Tsc]	Green	84%	493.06	-	240
[Pd(II)Ptol Tsc .cl]	Green	92%	493.06	28	221
[Pd(II)Dimet Tsc]	Red	96%	419.76	21	232
[Pd(II) Diet Tsc]	Brown	82%	436.82	24	254
[Pd(II)Diipr Tsc]	Green	96%	509.04	26	246
[Pd(II)Di phe Tsc]	Brown	92%	588.86	20	260

Nuclease Activity Studies:

The nuclease activity of present ligands and their - complexes has been investigated on pBR 322 plasmid DNA by agarose gel electrophoresis in the presence / absence of H₂O₂. At micro molar. concentration, the ligands exhibit no significant activity in absence and in the presence of the oxidant as shown in Fig 1. The nuclease activity is greatly enhanced by incorporation of metal ions in the ligands. In absence of oxidants , the Pd(II)-Complexes of BTsc and Substituted Thiosemicarbazones cause discernible DNA cleavage as evidenced by increase in intensity in form II (nicked) and form III (linear) with decrease in intensity in from I (super coiled) which is attributed to step-wise conversion of from I to form II and to form III. Similar observations were also evident in the Palladium(II)-Complexes of BTSC and Substituted TSCs. The nuclease activity of the Pd (II) complexes with BTsc is more.

All complexes show much enhanced nuclease activity in the presence of oxidant, which may be due to free radical reaction (OH*) with DNA. The production of hydroxyl radicals due to the reaction between H₂O₂ and the metal complexes. The OH* radical involves oxidation of deoxyribose moiety followed by hydrolytic cleavage of sugar phosphate backbone^[36].

On the basis of physicochemical and spectral data the metal chelates are assigned to be Square Planar complexes.

The Formulae Of The complexes could be written as [M L].

Where M = Pd (II) and L=BTsc, B.MTsc, B.Et Tsc, B.Ipr .Tsc, B.Cyc.Tsc, B.Ptol.Tsc,B.Phe.Tsc,

B.Bnzy.Tsc, B.Otol. .Tsc, B .Dimethyl.Tsc, B .Diethyl .Tsc, B.Diisopr.Tsc.B.Diphenyl.Tsc .

CONCLUSIONS

In summary, we have synthesized ligands L=B.Tsc, B.MTsc, B.Et Tsc, B.Ipr .Tsc, B.Cyc.Tsc, B.Ptol.Tsc, B.Phe.Tsc, B.Bnzy.Tsc, B.Otol. .Tsc, B .Dimethyl.Tsc,B .Diethyl .Tsc, B.Diisopr.Tsc,B.Diphenyl.Tscs . mentioned above and their complexes with Palladium (II). All complexe's plausible structures are supported by LSI Mass spectral data along with physico chemical and IR, NMR,Mass,ESr,Electronic spectral data. The ligands and their complexes would be screened for their anticancer activity against certain cancer cell lines. We have developed a simple, convenient and effective method for the synthesis of complexes. To our knowledge, this is the first report of an efficient general method for the synthesis of different Palladium (II) complexes of Benzil Thiosemicarbazone and Substituted Thiosemicarbazones.

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