

ORIGINAL RESEARCH ARTICLE

Synthesis, Characterization and Nuclease Activity of Au(III)- Complexes of Biacetyl Monoxime Thiosemicarbazone(BAMOT) and Substituted Thiosemicarbazones.**Prakash.MMS.Kinthada*¹ and M.Adharvanachary*²**¹Department Of Chemistry, GIT,GITAM University, Rushikonda, Visakhapatnam, 530045.INDIA.²Department Of Complexity Science and Engineering, Division Of Trans Disciplinary Science, University Of Tokyo, JAPAN.

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ABSTRACT

Metal complexes of Au(III) with Thiosemicarbazone and Substituted Thiosemicarbazones of different thiosemicarbazones have been synthesized. The present communication reports the structural study of the Au(III) complexes with Biacetyl Monoxime Thiosemicarbazones and these complexes have been characterised by elemental analysis, conductance, IR, NMR and Mass spectral data. The physico chemical and spectral data suggests tetrahedral geometry for various complexes. Metal complexes of Biacetylmonoxime thiosemicarbazone (BAMOT) and Substituted Thiosemicarbazones are also investigated for better comparison. Au(III) complexes of Biacetylmonoxime thiosemicarbazone (BAMOT). The ligand and metal chelates would be screened *in-vitro* for anticancer activity against some cancer Cell lines.

Key words: AU(III) complexes, BAMOT, Anticancer Activity.**INTRODUCTION**

Investigations of novel transition metal complexes to probe nucleic acids are the focus of current research. Thiosemicarbazones are biologically active pharmacophores, besides having good complexing ability and their activity enhances on complexation with metal ions^[1-4]. Thiosemicarbazone metal chelates have broad applications in biological and industrial fields^[5-13]. These complexes are known to be biologically important for antimicrobial^[5-6], antibacterial^[7-8], antifungal^[9-10], antitumour^[11]. Thiosemicarbazone and their metal chelates find important applications in the fields like pharmacology as well as medicine^[14-15]. It is observed that biological activity of ligand increases on complexation with different metal ions. Metal complexes of ligands containing both oximes and thiosemicarbazones are pharmacophores much less investigated. Although oximes and their transition metal complexes have been investigated as chemical nucleases^[10]. Metal complexes of ligands containing both oximes and thiosemicarbazones are pharmacophores much

less investigated, especially the Au(III) complexes of Thiosemicarbazones have never been reported and we report a series of Gold(III)-Biacetyl Monoxime Thiosemicarbazone complexes for the first time Metal compounds of diacetylraonoxime thiosemicarbazone (BAMOT) have been characterized but their nuclease activity has not been investigated so far. Recently the nuclease activity of copper complexes of ortho substituted heteroaromatic thiosemicarbazones and semicarbazones has been investigated^[16-18]. Hence we also report the Nuclease activity studies of the Gold(III) complexes of BAMOT and BAMOT-Substituted Thiosemicarbazones for the first time.

EXPERIMENTAL

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 analyses were performed by using micro analytical techniques. Gold (III) was estimated by using AAS model Z-6100 (Hitachi Ltd.). Chlorine is estimated by using standard procedures. The IR spectra were recorded in the range $4000-200\text{cm}^{-1}$ using KBr discs with Perkin-Elmer model 1430 and 337. The electrical conductivity measurements were made in DMF (10^{-3} M) at room temperature ($27 \pm 2^{\circ}\text{C}$) using a Digisun digital conductivity meter (DI-909 model). The NMR spectra were recorded in DMSO- d_6 on NMR spectrophotometer model JEOL Ex-90 FT using TMS as the reference. Mass spectrometer operating under liquid secondary ion mass spectral (LSIMS) conditions. 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 ISSf vibrating sample magnetometer operating at field strength of 2-1.0 KG.

Synthesis of Ligands:

In a clean 100ml round bottom flask, the reaction mixture containing Biacetylmonoxime ($5 \times 10^{-2}\text{M}$) in 100 ml of 1% Ethanol, thiosemicarbazide ($5 \times 10^{-2}\text{M}$) dissolved in boiling water were taken and refluxed for 3 hrs on cooling a white product for Biacetyl monoxime thiosemicarbazone was formed. It was collected by filtration, washed several times with hot water, small quantities of cold methanol and dried in vacuo. BAMOT-Substituted thiosemicarbazones were prepared according to literature reports,^[11-13] The pK_a values calculated by Philips-Merritt method is 8.55.

Synthesis of metal complexes:

The metal, complexes were prepared by mixing hot ethanolic solution of Au Cl₃ and BAMOT in the molar ratio of 1:1. In the preparation of Gold(III) complex, an aqueous solution of AuCl₃ was used. Metal solution was added to the boiling solution of ligand (2 g, 0.0084 moles) in ethanol and heated under reflux for 3 h. The reaction mixture was cooled and left overnight in refrigerator. Crystalline complexes, which separated out, were collected by filtration, washed with distilled water and small quantity of cold methanol.

Plasmid isolation:

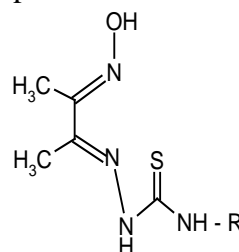
The E. coli DH5alpha strains containing plasmid pBR 322 was grown in Luria broth (LB) medium supplemented with 100 Micro g/ml ampicillin. Cells from 5 ml culture were harvested by centrifuging the culture for 10 minutes at 8000 rpm. Plasmid pBR 322 was isolated using Qiagen column following manufacturer's 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 wetter and plasmid bands were visualized by viewing the gel under transilluminator and photographed [19].

RESULTS AND DISCUSSION

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 (Table1) of the Gold (III)- BAMOT and Substituted BAMOT's indicates that all the metal complexes are non electrolytes [21] 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.



R = H (BAMOT), CH₃ (MBAMOT), C₂H₅ (MBAMOT), i-Pr (MBAMOT), Cy.Hex. (MBAMOT), p.Tol (MBAMOT), C₆H₅ (MBAMOT), Benzy (MBAMOT).

The present ligands contain two functional groups viz. oxime and thiosemicarbazone (Structure I) 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 :

Table 1: Physical and analytical data of the Au(III) complexes of BAMOT and Substituted BAMOT's

S.No	Ligand/Complex	Colour	Yield	Mol.Wt	Conductance Ohm-1.cm ² .mol.	M.P ^o C
1	[Au(BAMOT)] ₂ Cl ₂	Yellow	80%	667.42	38	162
2	[Au(MBAMOT)] ₂ Cl ₂	Yellow	85%	682.42	45	168
3	[Au(EBAMOT)] ₂ Cl ₂	Brown	90%	696.44	36	182
4	[Au(IPBAMOT)] ₂ Cl ₂	Yellow	78%	710.42	52	194
5	[Au(CyBAMOT)] ₂ Cl ₂	Brown	92%	739.44	44	208
6	[Au(PhBAMOT)] ₂ Cl ₂	Red	88%	743.46	48	234
7	[Au(Benz BAMOT)] ₂ Cl ₂	Yellow	94%	759.48	42	182
8	[Au(PtolBAMOT)] ₂ Cl ₂	Yellow	82%	758.44	36	196

Infra Red Spectra:

The important vibrational bands of metal complexes are included in Table 3. The absence of SH band at 2570 cm⁻¹ and presence of NH band at 3233 cm⁻¹ in the IR spectrum of ligands suggest that the ligands remain in thione form at least in solid state. The infrared spectrum of BAMOT shows bands at 3144, 1602 and 1193 cm⁻¹ assigned to $\nu(\text{GH})$ of oxime, $\nu(\text{C}=\text{N})$ and $\nu(\text{C}=\text{S})$ respectively. The Ligand IR bands around at 3380 and 3280 cm⁻¹ are assigned to the asymmetric and symmetric stretching of free NH₂ group. These bands remained unaltered in the spectra of the complexes which indicate non-participation of NH₂ in coordination [19]. A strong band appearing at 1165 and 1124 cm⁻¹ in the spectra of BAMOT, MBAMOT and other BAMOT-Substituted Thiosemicarbazone complexes with Au(III) is shifted to lower frequency indicating the involvement of thioketo sulphur in coordination. A strong band observed at 3416 and 3406 cm⁻¹ in the IR spectra of BAMOT and MBAMOT and other complexes disappeared in the spectra of all complexes suggesting deprotonation of oxime OH in the complex formation. The $>\text{C}=\text{N}$ (imine band) is observed at 1600 and 1605 cm⁻¹ in the IR spectra of BAMOT, MBAMOT and other complexes respectively. These peaks are shifted by 10–20 cm⁻¹ in the metal chelates indicating coordination through azomethine nitrogen [20]. A strong band appearing at 1186 and 1126 cm⁻¹ in the spectra of BAMOT is shifted to lower frequency, indicating the involvement of thio-keto sulphur in coordination. A strong band observed at 3406 and 3408 cm⁻¹ in the IR spectra of PPDOT and DAMOT disappeared in the spectra of all complexes suggesting deprotonation of oxime OH in the complex formation. The $>\text{C}=\text{N}$

(imine band) is observed at 1600 in BAMOT. This band is shifted to lower wave numbers in the spectra of complexes suggesting the participation of imine nitrogen atom in coordination. Additional bands are observed in Far IR spectra of metal complexes in 500-480 and 365-315 cm⁻¹ regions due to $\nu(\text{M}-\text{N})$ and $\nu(\text{M}-\text{S})$ modes respectively. Based on molecular weight determination, magnetic moments, electronic and IR spectra a general structure (Structure I) is assigned for the complexes.

NMR Spectra:

The study of the NMR spectrum of Gold(III) metal chelates illustrates the presence of aromatic protons (benzimidazolyl moiety), NH₂ protons of thiosemicarbazide moiety, N-H iminoprotons and C-methyl protons. The NMR spectrum of ligand shows different signals at δ 10.40 (imino protons), 8.2 and 7.12 ppm aromatic proton (peri), δ 7.50 ppm (NH₂ protons) of thiosemicarbazide moiety [13] and a singlet at δ 2.46 ppm corresponds to C-methyl. These signals are not changed in the nmr spectra of the metal complexes. The ¹H-NMR spectrum of BAMOT was recorded in 4-DMSO solvent. It shows signals corresponding to -CH₃, -NH₂, NH (hydrazone) and -OH protons at 2.13 (s, 3H), 7.18-7.48 (m, 5H), 8.70-8.10 (2H), 10.64 (s, 1H) and 11.70 (s, 1H) respectively. The nmr spectrum of metal chelates confirms the non participation of NH₂ group and imino NH group in the coordination with metal ions.

Mass Spectra:

The mass spectrum of ligand and metal complexes is recorded under liquid secondary ion mass spectral conditions [21-23]. The ligands BAMOT and MBAMOT gave the peaks at m/z 162 and 173 Da₁ and these values confirm the molecular weight of the ligands. The LSIM spectra of the

complexes of BAMOT ligands showed abundant ions at m/z 186 and 568, 573, 668, 842 corresponding to $[(L-H)]^+$ and $[(2L+2M-2H)]^+$ ions. The ions supports $[ML]_2X_2$ composition.

Electron spin resonance spectra:

Electronic spin Resonance spectra of Gold complex was recorded in DMF at liquid nitrogen temperature value equal to 20.

ronic spin Resonance spectra of Gold complex was recorded in DMF at liquid nitrogen temperature.

Electronic Spectra:

Since All the Gold(III) complexes are Diamagnetic, they did not show any bands in electronic spectrum.

Magnetic Studies:

All the complexes are diamagnetic.

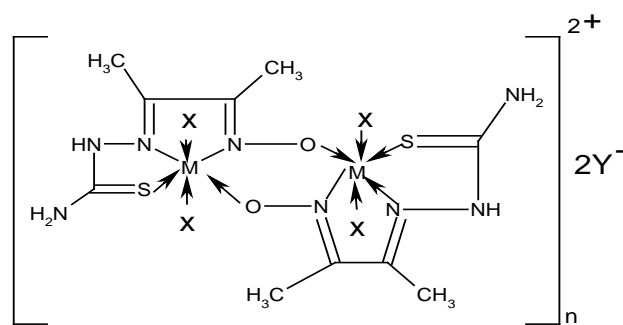
Molar Conductance studies:

The molar conductivity data suggest that the complexes are 1:1 electrolytes. 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.

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 H_2O_2 . 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 m the . ligands. In absence of oxidants , the Gold(III)-Complexes of BAMOT's causes 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 Gold(III)-Complexes of BAMOT and the conversion to linear form was complete. The . nuclease activity of the Gold(III) complexes with BAMOT is more when compared to other Metal Complexes of BAMOT.

On the basis of physicochemical and spectral data the metal chelates plausible structure I may be given as follows. Tetrahedral geometry is suggested for all the Au(III) complexes.



Structure I

M = Au(III) ;

L=BAMOT.Tsc, BAMOT.MTsc, BAMOT.Et Tsc, BAMOT.Ipr .Tsc, BAMOT.Cyc.Tsc, BAMOT.Ptol.Tsc BAMOT.Phe.Tsc, BAMOT.Bnzy.Tsc .

The Nuclease activity of of present Ligands and their complexes has been investigated on pBR 322 plasmid DNA by Agarose Gel Electrophoresis in the presence/Absence and in the presence of Oxidant.The Nuclease activity is grately enhanced by incorporation of Metal Ion in the ligands. In the absence of Oxidants Au(III) complexes of BAMOT and Substituted BAMOT's cause Discernible DNA cleavage as was evidenced increase in form-II(Nicked) and form-III(linear) with decrease in intensity in form-I(super coiled) which is attributed to mstepwise conversion of form-I to form-II and to form-III.

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 H_2O_2 and the metal complexes.The OH^* radical involves oxidation of deoxyribose moiety followed by hydrolytic cleavage of sugar phosphate backbone[21].

The higher activity of BAMOT complexes is probably due to presence of lipophilic $-CH_3$ group. The lipophilic nature is evaluated, by thin layer L chromatography for the ligands in $10^{-3} M$ alcoholic solutions. The stationary phase was silica gel chemically bonded (Nano-Si NH_2) and mobile phase was a 2:3 mixture of water and methanol. The R_f values[BAMOT – around 46in mm] indicate more lipophilic nature of BAMOT and its complexes.

In summary, we have synthesized ligands BAMOT, MBAMOT and other substituted Thiosemicarbazones mentioned above and their complexes with Gold(III). All complexes' plausible structures are supported by LSI Mass spectral data along with physico chemical and IR, NMR spectral data. The ligands and their complexes were screened for their antifungal and antibacterial activity against some of the pathogenic fungi and bacteria and observed that the metal complexes are showed more biological activity than ligands. 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 and general method for the synthesis of different metal complexes.

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