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SYNTHESIS, CHARACTERIZATION AND NUCLEASE ACTIVITY OF AU (III) - COMPLEXES OF BIACETYL MONOXIME THIOSEMICARBAZONE (BAMOT) AND SUBSTITUTED THIOSEMICARBAZONES

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ABSTRACT

Metal complexes of Au(III) with Thiosemicarbazone and Substituted Thiosemicarbazones 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 characterized by elemental analysis, conductance, IR, NMR and Mass spectral data. The physicochemical and spectral data suggests tetrahedrol geometry for various complexes. Metal complexes of Biacetyimonoxtme thiosemicarbazone (BAMOT) and Substituted Thiosemicarbazones are also investigated for better comparison. Au(III) complexes of Biacetyimonoxtme thiosemicarbazone (BAMOT) and Substituted Thiosemicarbazones are also investigated for better comparison. The ligand and metal chelates would be screened *in vitro* for anticancer activity against some cancer Cell lines.

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], and antitumor [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 Thiosemicarbazonbes have never been reported and we report a series of Gold(III)-Biacetyl Monoxime Thiosemicarbazone complexes for the first time.. Metal compounds of diacetylraonoxime thiosemiearbazone (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 regent 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. 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 4000-200cm^{-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^{o}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 was operated 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 (5x10-2M) in 100 ml of 1% Ethanol, thiosemicarbazide (5x10-2M) dissolved in boiling water were taken and refluxed for 3 hrs. On cooling a white product for Biacetyl monoxime thiosemicarbazone was formed. 1t 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* DH5-alpha strains containing plasmid pBR 322 was grown in Luria broth (LB) medium supplemented with 100µg/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.

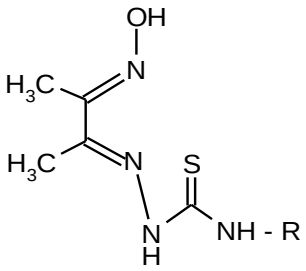
Assay of Nuclease Activity: The DMF solution containing metal complexes was taken in a clean eppendroff tube and 1 micro gram of plasmid DNA was added. The contents were incubated for 30 min at 37°C and loaded on 0.8% agarose gel after mixing 5 micro liters of loading buffer (0.25% bromophenol blue + 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 H_2O). 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 (**Table 1**) 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), CH_3 (MBAMOT), C_2H_5 (MBAMOT), i-Pr (MBAMOT), Cy. Hex. (MBAMOT), p. Tol (MBAMOT), C_6H_5 (MBAMOT0, Benzy(MBAMOT)

The present ligands contain two functional groups viz. oxime and thiosemicarbazone (Structure I) and their metal complexes are stable at room temperature, hygroscopic, insoluble in H_2O, but slightly soluble in ethanol and methanol and readily soluble in DMF and DMSO. The color, molecular weight and molar conductance data are summarized in **table 1.**

TABLE 1: PHYSICAL AND ANALYTICAL DATA OF THE Au(III) COMPLEXES OF BAMOTAND SUBSTITUTED BAMOT's

Ligand/Complex	Color	Yield	Mol. Wt	Conductance ($Ohm^{-1}.cm^2.mol^{-1}$)	M. Pt. ^oC
$[Au(BAMOT)]_2Cl_2$	Yellow	80%		38	162
[Au(MBAMOT)]2cl2	Yellow	85%		45	168
[Au(EBAMOT)]2cl2	Brown	90%		36	182
[Au(IPBAMOT)]2cl2	Yellow	78%		52	194
[Au(CyBAMOT)]2cl2	Brown	92%		44	208
[Au(PhBAMOT)]2cl2	Red	88%		48	234
[Au(Benz BAMOT)]2cl2	Yellow	94%		42	182
[Au(PtolBAMOT)]2cl2	Yellow	82%		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"' 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 v(GH) of oxime, v(C=N) and v(C=S) respectively. The Ligand IR bands around at 3380 and 3280 cm^{-1} are assigned to the asymmetric and symmetric stretching of free NH_2 group. These bands remained unaltered in the spectra of the complexes which indicate non-participation of NH_2 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 >C = 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 > C = N (imine band) is observed at 1600in 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 v(M-N) and v(M-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), NH_2 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), δ7.50 ppm (NH_2 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 ^{1}H-NMR spectrum of BAMOT was recorded in 4-DMSO solvent.

It shows signals corresponding to -CH_3, -NH_2, 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 NH_2 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 are gave the peaks at m/z 162 and 173 Da_1 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.

Electronic Spectra: The Gold(III) complexes show peaks in the electronic spectrum at 14850 and 19130cm-1 corresponding to d-d transitions and two bands at 29,3000 cm-1 assignable to L to M charge transfer.

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**.

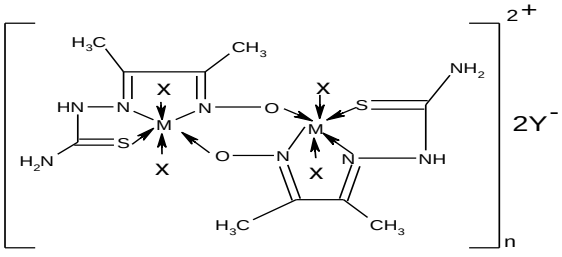


FIG. 1:

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 is greatly enhanced by incorporation of metal ions *m* in 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 11 (nicked) and form III (linear) with decrease in intensity in from 1 (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 of value 20.

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 and in the presence of Oxidant. The Nuclease activity is greatly 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(linier) with decrease in intensity in form-I(super coiled) which is attributed to stepwise 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 $OH^{\cdot}$ 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 –CH3 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 NH2) and mobile phase was a 2:3 mixture of water and

methanol. The Rf 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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REFERENCES:

1 Mookerjee M N, Singh R V & Tandoti J P, *Tmns Met Chem,* 10 (1985)66.
2 Antholine \V F. Knight J M *&* Petering D H. *J Med Chem,* 19 (1976)329
1. Peirin DD & .Siunzs H, in *Metal kms in Biological Systems.* edited by ii Sigel (Marcel Dekker. New York); pp. 207.
2. Ligappa Y & tfussain Reddy K, *Indan J Chem.* 33A (1994) 919
3. 35 A (1996) 775.
4. N.S. Pawar, D.S. Dalal, S.R. Shimpi, Eur. J. Pharm. Sci. 21 (2004) 115.
5. G. Ayhan, N. Altanlar, Il Farmaco 58 (2003) 1345.
6. Y. He, B. Wu, J. Yang, Bioorg. Med. Chem. Lett. 13 (2003) 3253.
7. V.K. Limesova, J. Koci, K. Waisser, Il Farmaco 57 (2002) 259.
8. D.A. Horton, G.T. Bourne, M.L. Smythe, Chem. Rev. 103 (2003) 893.
9. H. Kucukbay, R. Durmaz, E. Orhan, Il Farmaco, 58 (2003) 431.
10. A.A. Spasov, I.N. Yozhitsa, L.I. Bugavea, J. Pharm. Chem. 33 (1999) 232.
11. J.S. Casas, M.S. Garcia-Tasende, J. Sordo, Co-ord. Chem. Review 213 (2001) 331.
12. M. Ali, M. Nizamuddin, F. Smith, G. Hynes, Polyhedron 15 (1996) 975.
13. Avinash K, Shirish D, Prasad K. Duduka S & Chatar V, *Ind.J.Chem, 35A (1996)533.*
14. *Chem.* 35A (1996) 533.
15. Nigani S, Pate! *M* M *&* Raj- A, *Synth Reactin inorg. Organomet.chem,* 28(1998)182.
16. Hussain Reddy K. Sambasiva Reddy P & Ravindra Babu P, Trans.Met.Chem.25 (2000)154.
17. Hussain Reddy K. Sambasiva Reddy P & Ravindra Babu P,*Trans.* Met. ChemJ.Inorg.Biochem,7(1999)169.
18. Hussain Reddy. K, Sambasiva Reddy. P and Ravindra Babu.P, Trans.Met.Chem25 (2000), 505.
19. Maniatis T, Frit.sch E F *&* Sambrook*. Molecular Cloning. A Laboratory Manual,* (Cold spring Harbor, Lab, Press,Plain view, NY; 1990. p, 149-172.
20. K. Syamasundar, M. Adharvana chari, J. Indian Chem. Soc. 78 (2001) 32.
21. Yamamoto K& Kawanishi, J.Biol.chem, 264(1984)15.

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