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ORIGINAL RESEARCH ARTICLE

Synthesis, Charecterization and Nuclease Activity of Au(III)- Complexes of Alloxan Thiosemicarbazone (All.Tsc) and Substituted Thiosemicarbazones.

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ABSTRACT

Abs**tract:** Some Hitherto unknown complexes of Alloxan 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 Au(III) complexes are tetrahedral.The ligand,its Schiff's Bases and and metal chelates would be screened *in-vitro* for anticancer activity against some cancer Cell lines.

Key words: Alloxan Thiosemicarbazone, Au(III), ligand, Schiff's Bases, metal chelates

INTRODUCTION

The Thiosemicarbazide and Thiosemicarbazone compounds have gained specia;l attention due to their activity against Protozoa[1], influenza[2], small pox[3] and certain kind of tumors[4,5].Since Domagk's original discovery[6]of their cAntitubercular Activitythe number of papers on the pharmacology of these compounds has increased dramatically.A large number of Thiosemicarbazones have been evaluated for their Antimalarial, Antitumor and and Antibactwerial activity because of their useful mchemotheraupetic properties[7].In cancer treatement, it has been shown that the Metal chelates are more potent than chelating agents[8,9].Thiosemicarbazones and substituted thiosemicarbazones have been found to be more effective in reducing tissue Iron levels than the Deferroxamine , which is used for removing excess Iron accumulated in the tissues of patients with cooley's anemia[10].They have been employed cas chelating agents for Technetium-99 labelling proteins[11].A thorougfh survey of the literature reveals that only a few reports have appeared on the synthesis, characterization and biological activity of Mretal complexes of substituted Thiosemicarbazones, especiall Gold.We recently reported for the first time some Gold (III) complexes of Biacetyl Monoxime Thiosemicarbazones[12] .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[19-22].Thiosemicarbazone metal chelates have broad applications in biological and industrial fields[13-22]. Thiosemicarbazone and their metal chelates find important applications in the fields like pharmacology as well as medicine[23-24]. Au(III)-complexes of Alloxan Thiosemicarbazones have not been investigated until now. Hence we report a series of Gold(III)-Alloxan Thiosemicarbazone/s complexes for the first time. Apart from studying the Metal compounds of Alloxane Thiosemicarbazone, their nuclease activity has not been investigated .Recently the nuclease activity of copper complexes of ortho substituted heteroaromatic thiosemicarbazones and semicarbazones has been investigated[25-27]. Hence we also report the Nuclease activity studies of the Gold(III) complexes of Alloxan Thiosemicarbazone and substitutexd 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

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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 procedure. 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 ± 2^0c) 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. 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 polycrystall ine 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 Alloxan 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 3oml of Au(III) solution (5x10-2M) in Methanol was added (1x10-2M) ATsc solution in methanol and the mi8xture refluxed for abot 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 H_20). The gel was then de-stained for 10 min by keeping it in sterile distilled weter and plasmid bands were visualized by viewing the gel under transilluminator and photographed [17].

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 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 amd 740cm-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 , 380-300 cm-^{1} and 270-260 cm-1 regions due to **v(M-N),** v(M-S) and v(M-Cl) modes respectively. Based on molecular weight determination, magnetic moments, electronic and IR spectra a

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general structure (Structure I) is assigned for the Alloxane 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 8.2 and 7.12 ppm aromatic proton , δ7.50 ppm (NH_2 protons) of thiosemicarabazide moiety[13] . These signals are not changed in the nmr spectra of the metal complexes. The H-NMR spectrum of All.Tsc was recorded in 4-DMSO solvent. It shows signals corresponding to - -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 in the coordination with metal ions.1H NMR signals are well defined and the spectrum of free ligand [29] exhibits two resonances for the NH2 protons at 7.5 ppm ,a result explained in terms of hindered rotation about the C(S)- 4NH2 bond due to its partial double bond character [30,31]. The metal complexes show only one resonance due to 4NH2 protons , upfield for some complexes (at 9.41 ,8.28 ,8.19) and down field (at 9.68) for some complexes .The signal at 11.85 ppm in the spectrum of ligand due to 2'NH is present in most of the complexes with down field shifts (11.36 ,11.39, 11,58) and with an upfield shift for one complex (12.36) 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 (Table1) of the Gold (III)-Alloxan thiosemicarbazone and Substituted BAMOT's 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[21-23].The ligands All.Tsc and Sust.Tscs gave the peaks at m/z 164 and 176 Da_1 and these values confirms 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 supports the proposed composition and structure.

Electron spin resonance spectra:

Electronic spin Resonance spectra of Gold complex was recorded in **DMF** at liquid nitrogen temperature.value equal to 26.

Electronic Spectra:

Since all the Au(III) complexes arre diamagnetic, the electronic spectra did not show any bands.

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.

The Alloxane 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 Au(III) complexes of Alloxan Tsc and Substituted Tscs

S.No	Ligand/Complex	Colour	Yield	Mol.Wt	M.P^{0}C
1	[Au(All.Tsc.cl)]	Brown	88%	201.08	246
2	[Au(All.Me Tsc.cl)]	Brown	94%	216.08	238
3	[Au(III) Et.AllTsc .cl]	Brown	96%	230.04	256
4	[Au(III).Ip All Tsc .cl]	Red	88%	244.06	264

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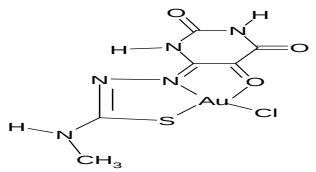
5	[Au(III)Cy All.Tsc .cl]	Brown	94%	274.04	248
6	[Au(III) Ph All Tsc.cl]	Red	88%	278.02	230
7	[Au(III) Benz All.Tsc.cl]	Brown	92%	292.04	248
8	[Au(III) Otol. All .Tsc .cl]	Green	86%	305.4	236
9	[Au(III) Ptol All .Tsc .cl]	Green	88%	305.4	226
10	[Au(III) Dimet. All .Tsc .cl]	Red	94%	231.06	246
11	[Au(III) Diet .All .Tsc.cl]	Brown	84%	261.06	262
12	[Au(III) Diipr All .Tsc .cl]	Green	94%	1277.2	256
13	[Au(III) Di phe. All .Tsc .cl]	Brown	90%	355.06	268

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 *m* the . ligands. In absence of oxidants , the Gold(III)-Complexes of All.Tsc and Substituted Thiosemicarbazones cause discernible DNA cleavage as evidienced 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 All.Tsc and Substituted Tscs. The . Nuclease activity of the Gold(III) complexes with All.Tsc 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 H202 and the metal complexes. The OH* radical involves oxidation of deoxyribose moiety followed by hydrolytic cleavage of sugar phosphate backbone[32].

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.



M = Au(III)

L=All.Tsc, All.MTsc, All.Et Tsc, All.Ipr .Tsc, All.Cyc.Tsc, All.Ptol.Tsc,All.Phe.Tsc, All.Bnzy.Tsc, All.Otol. .Tsc, All .Dimethyl.Tsc, All .Diethyl .Tsc, All.Diisopr.Tsc.All.Diphenyl.Tsc .

CONCLUSION

In summary, we have synthesized ligands L=All.Tsc, All.MTsc, All.Et Tsc, All.Ipr .Tsc, All.Cyc.Tsc,All.Ptol.Tsc,All.Phe.Tsc,All.Bnzy.Tsc,All.Otol.Tsc,All .Dimethyl.Tsc, All.Diethyl.Tsc, All.Diisopr.Tsc, All.Diphenyl.Tscs mentioned above and their complexes with Gold(III). 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 Au(III)complexes of Alloxane Thiosemicarbazone and Substituted Thiosemicarbazones.

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