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Anthraquinone Benzylthiosemicarbazone Cr (III) Complex as a Potential Anti-Cancer Drug-Characterization and Activity

J. Sai Chandra[a], Y.A.S.J. Prasanna Kumari[a], P.N.V.V.L. Prameela Rani[a], Y. Sunandamma[b]*

[a]*Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, A.P, INDIA-522510.*
[b]*Department of Chemistry, Vikrama Simhapuri University, Nellore, A.P, INDIA-524001.*
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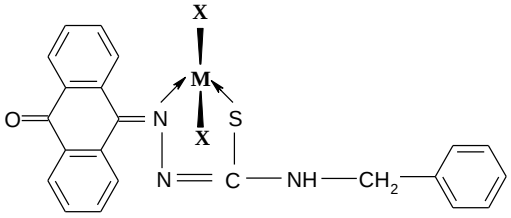
ABSTRACT
The suggestion that thiosemicarbazones act as iron chelators interfering with DNA synthesis to prevent their production and copper complexes with aromatic thiosemicarbazones affected the complicated mechanism of leukemic transformations, lead to a lot of interest in their metal complexation reactions. Provision of additional donor sites, improve their denticity and utilizing N^4 substitution on the ligand have become vital in their remarkable antitumor, antibacterial, antifungal activities. A novel Anthraquinone N^4benzylthiosemicarbazone Cr (III) complex with a potential to act as an anti-cancer drug has been synthesized and characterized by spectral methods. Effect of N^4-substitution, additional donor sites and complexation on the biological activity of the ligand has been studied.

Key words: *Substituted Thiosemicarbazides, Stoichiometr, Antibacterial Activity, Anti Cancer Drug*

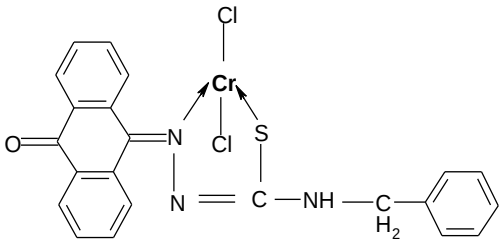
1. INTRODUCTION

Thiosemicarbazones are now well established as an important class of nitrogen and sulphur atom donor ligands particularly for transition metal ions [1-3]. This is because of remarkable biological activities observed for these compounds, which were shown to be related to their metal complexing ability. Thiosemicarbazone complexes present a great variety of biological activity ranging from anti-tumour, anti-fungal, anti-bacterial, anti-inflammatory to anti-viral activities [4-8]. The suggestion that thiosemicarbazones act as Iron chelators, interferes with DNA synthesis to prevent their production led to a lot of interest in their complexation as well as their pharmaceutical importance [9]. Copper complexes with aromatic thiosemicarbazones affected the complicated mechanism of leukemic transformations by inhibiting the replication and triggering apoptotic processes, proving themselves as potential antitumor agents [10,1]. At present, as far as their enhanced biological activities are concerned, majority of studies on transition metal complexes of thiosemicarbazones are two fold, a) Provide additional donor sites and improve their denticity, b). Utilize N^4 substitution to produce ligands with varied biological activities. These modifications extended the biological importance of thiosemicarbazones with added flexibility, selectivity and sensitivity towards metal ion complexation.

In spite of the fact that a large number of chemotherapeutics are available today for specific treatments, search for new drugs has become inevitable and is the order of the day due to anti-microbial resistance of some of the well-known drugs [12]. Metal ions play a major role and transition metal ions present a wide choice for taking up the required route in the development of new drugs.



(a)



(b)

Scheme I: Proposed structure of (a) Metal complex (b) Synthesized Anthraquinone N^4-benzyl 3-thiosemicarbazone Cr(III) complex.

Anthraquinones are known for their traditional therapeutic use in the form of plant extracts for skin disorders and for anticancer activity and exhibit

*Corresponding Author:
E-mail address: sunandamma@rediffmail.com

Table 1: Physical characteristic of ligand and the Aqn benzylTSC Cr (III) complex.

Compounds	Yield (%)	Colour	MP ^{o}C	% Yield
Ligand (Aq BenzylTSC)	95	yellow	250	90
Metal Complex (Aq BenzylTSC Chromium complex)	90	Partial Crystalline Light green	265	75

interesting in vivo biological activities. There has been a stronger focus in recent years on substituted ligands, serving an important purpose in complexation with metal ions. These observations led to our present work and we have synthesized Cr(III) complex of Anthraquinone N^4-benzylthiosemicarbazone and characterized the complex by FTIR, UV-VIS, ^{1}H NMR, Mass Spectrum and CHN analysis. A comparative study of the biological activity of ligand with complexation and without complexation with respect to inhibition duration has been carried out with a wide choice of bacterial species *Candida albicabs, Xanthomonas, Pseudoaureginosa, L. Calci, B. megeteium, S. aureus, E. Coli, L. Acidophilus, Enterococus, S. Mutans* to ascertain the effect of additional binding sites, N^4 substitution and complexation with metal ion on biological activity

2. EXPERIMENTAL

2.1. Materials

Thiosemicarbazide, Anthraquinone, benzyl amine, carbon disulfide, chromium(III) chloride were of AR grade, purchased from Hi Media and E. Merck, used as received. Methanol, ethanol and chloroform were purified by using standard procedures.

2.2. Synthesis of Metal Complexes

The complex has been synthesized in three stages
Stage I-Substitution of Thiosemicarbazide
Stage II-Synthesis of ligand
Stage III-Synthesis of metal complex

2.2.1. Stage I - Substitution of Thiosemicarbazide

For aromatic substituted thiosemicarbazide, the preparation method was from established procedures using amines as starting materials. [13].

2.2.2 Stage II-Synthesis of Ligand

Anthraquinone benzyl thiosemicarbazones (AqnBenzylTsc) were synthesized by condensing equimolar solutions 0.104gm (0.01M) of Anthraquinone and Thiosemicarbazides/4- substituted thiosemicarbazides 0.045 gm (0.01M) in methanol in the presence of few drops of acetic acid. This mixture was refluxed on water bath for 2hrs. It was filtered and the filtrate was concentrated to half the volume. On cooling the solution colored crystals were obtained, separated and recrystalised from ethanol.

2.2.3 Stage III - Synthesis of Metal Complexes

0.185g (0.01M) of Anthraquinone benzylthiosemicarbazone (AqnBenzylTsc) was dissolved in hot distilled water, cooled and then filtered and 0.133g (0.01M) of $CrCl_3.6H_2O$ was dissolved in acetone. These two solutions were mixed together with constant stirring and mixture was refluxed for about 3 hrs. Light green colored precipitate was obtained on cooling. The precipitate was filtered, washed with distilled water and dried. It was recrystallized from ethanol and dried in an electric oven at 115 ^{o}C.

3. RESULTS AND DISCUSSION

The complex thus synthesized was found to be partially crystalline, light green in colour and soluble in hot distilled water, acetone, DMF but insoluble in cold water. A comparison of melting points of ligand and complex indicates that complexation enhances the thermal stability of the ligand as shown in Table 1.

3.1 FTIR Spectra

FTIR spectra were recorded using KBr pellets on Thermo Nicolet 6700 FT-IR spectrophotometer in the region 400-4000cm^{-1}. Ligand (AqBenzylTSC) displayed a band in the region 1692 cm$^{-1,}$ which was shifted from 1704 cm^{-1} in Anthraquinone, was assigned for quinonic carbonyl ν(C=O) group. Band located at 1590 cm^{-1} was attributed to ν(C=N), indicating substitution at N^1 of the thiosemicarbazide part of the ligand. ν(C=S) appeared at 819 cm^{-1} along with ν(N-H) at 3432 cm^{-1} in the ligand. ν(C-H) and δ C-H) were assigned at 1168 cm^{-1} and 938 cm^{-1} in the ligand along with all other characteristic bands for thiosemicarbazone ligands Table 2 and 3. In the case of Cr(III) complex, reduced (C=O) group frequency at 1685 cm^{-1}, ν(C=N) at 1585 cm^{-1} and ν(C=S) at 809 cm^{-1} indicate metal binding modes of Cr(III)ion with the ligand in the complex (Fig 1 and Fig.2) This may be due to coordination of the ligand to the metal ion through S-atom [14].

3.2 Optical Absorption Spectrum

The optical absorption spectrum of the ligand (Aqn benzylTSC) and complex (Fig.3) were measured by JASCO V670 spectrophotometer from 200 nm to 1400 nm. The spectrum of Chromium metal complex contains three absorption bands centered

Table 2: FTIR spectrum of Aqn benzylTSC Cr(III) complex.

S.No	Functional Group	Assignment		
		Aqn	Aqn BTSC	Cr(III)
1	C=O	1704	1692	1685
2	Aromatic ring C-H stretch	2964	2958	2948
3	C=N	-	1590	1585
4	C=S	-	819	
5	C-S	-	-	809
6	N-H	-	3432	3420
7.	Aromatic CH_2	-	2925	2906
8.	M-N	-	-	445
9.	Overtones and bands	-	1384,1304	1328
10	C-H in plane bending of Aqn	-	-	1282
11	C-H stretching	-	1168	1164
12	C-H bending	-	938	935

Table 3: Elemental analysis of CHN for the ligand and the Aqn benzylTSC Cr(III) complex**.**

Compound	Colour	Elemental analysis Found (Calcd) %		
		C	H	N
Ligand (Aqn Benzyl TSC)	Yellow	71.15(71.19)	4.58(4.61)	11.32(11.38)
Aqn Benzyl TSC Chromium complex	Partial crystalline Light green	71.45(71.41)	5.12(5.15)	10.96(10.99)

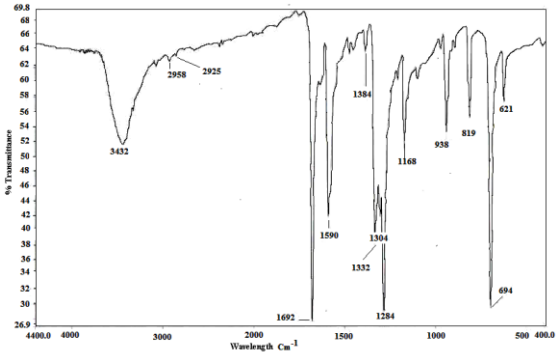


Figure 1: FTIR spectrum of 4-benzyl-3-thiosemicarbazone Anthraquinone.

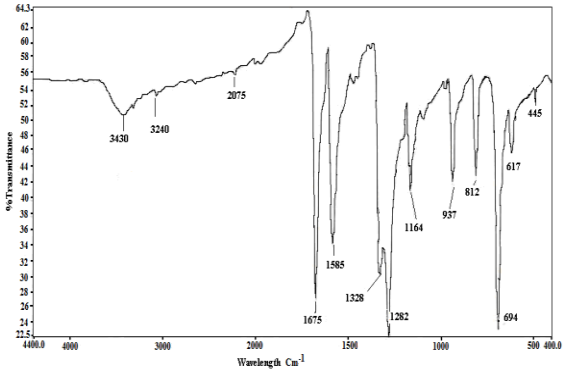


Figure 2: FTIR spectrum of Aqn benzylTSC Cr(III) complex.

at 205nm, 240nm and 320nm, which may be assigned to π→π* transition, n→π*(I) & n→π*(II).The π→π* transition in uncomplexed Chromium ion was retained at 205nm in the complex, which indicates the absence of coordination through the double bond of the ligand. The presence of n→π*(I) & n→π*(II) transitions indicate the coordination of the ligand through S-atom of the thiocarbonyl group of TSC ligand [15-17].

3.3 ^{1}H NMR Spectrum

The proton magnetic resonance spectrum of the complex, Aqn benzylTSC Cr(III) was recorded in DMSO solvent and measured on on BrukerAV400NMR Spectrometer using TMS as an internal standard. were shown in Fig.4. Actual signal positions along with coupling constants were assigned as follows :(s, 11.235 ppm), (s, 8.163 ppm), (s, 7.814 ppm), (s, 7.553 ppm), (s, 7.231 ppm), (s, 6.974 ppm), (s, 6.752 ppm), (s, 3.179 ppm), (s, 2.665 ppm), Hf (s, 2.946 ppm). Hg (s, 1.919 ppm). Hh (s, 1.163 ppm). Hi (s, 0.748 ppm) Table-4. In Aqn benzylTSC Cr (III) complex, signal at δ11.00 was assigned to N (1)-H based on the position of the peak in the spectrum of the parent thiosemicarbazide molecule. Aromatic proton exhibit signals in the region δ 8.4 ppm-δ 6.5 ppm [18].

3.4 Mass Spectrum

The electronic impact mass spectrum of the ligand Aqn benzylTSC (Fig 1) recorded on JOEL JMS-D 300Mass spectrometer and showed a series of peaks at 60, 88, 150, 178, 203, 241, 268, 280, 297,353 and 371 amu, corresponding to various

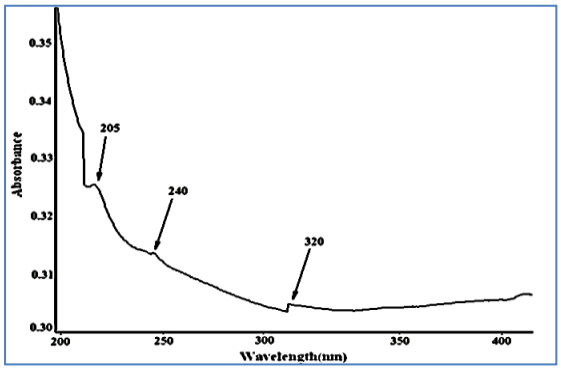


Figure 3: Optical Spectrum of complex Aqn benzylTSC Cr(III) Complex.

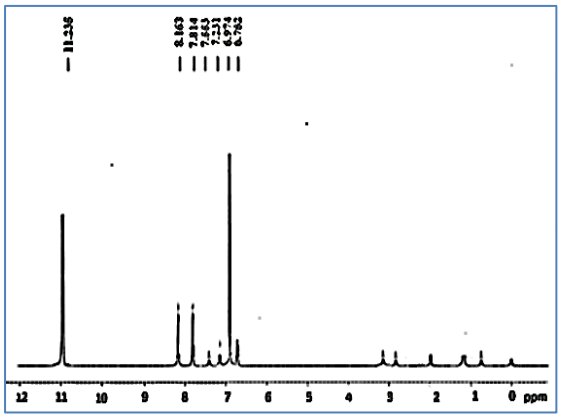


Figure 4: ^{1}HNMR Spectrum of complex Aqn benzylTSC Cr(III) Complex.

fragments in the ligand. The intensities of these peaks are related to the stabilities of the fragments. Aqn benzylTSC Cr(III) complex showed a single peak at 511 amu, which coincides with that of molecular ion. Loss of two chloride ions is in agreement with a peak at 476 amu. Loss of a part of the ligand was in agreement with a peak at 315 amu. A single peak at 371 amu coincides with that of ligands shown in (Fig 5, Fig.6). By this we concluded the proposed structure of complex.

3.5 Biological Activity and Discussion

The antibacterial activities of both ligand (Aqn benzylTSC) and its metal complex were studied by usual cup-plate agar diffusion method (Fig.7). The gram negative bacterial species used in the screening were *Candida albicans, Escherichia coli, Xanthomonas and Pseudo aureginosa*. The gram positive bacterial species used are *Lactobacillus calci, Bacillus megaterium, Staphylococcus aureus and Enterococus, L. Acidophilus* and *Streptococus mutans*. Stock cultures of the test bacterial species were maintained on Nutrient Agar media by sub culturing on Petri dishes. The media were prepared by adding the components as per manufacturer's instructions and sterilized in the autoclave at 121^{o}C temperature and 15 lbs pressure for 15 minutes and

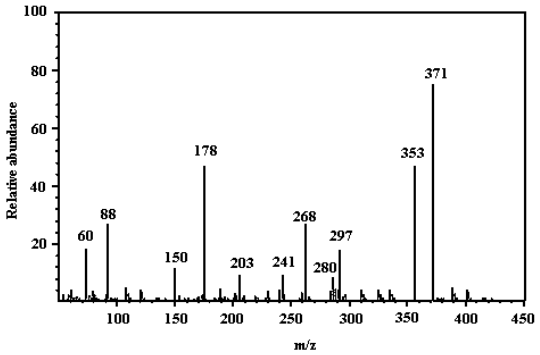


Figure 5: Electronic impact Mass Spectrum of Ligand (Aqn benzylTSC).

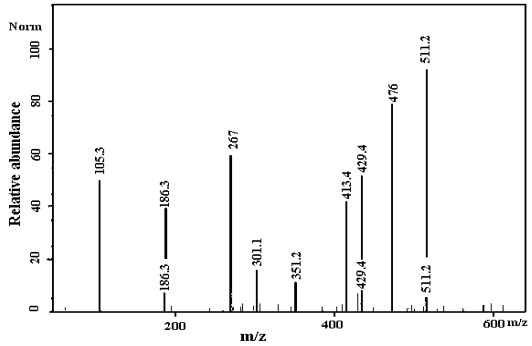


Figure 6: Electronic impact Mass Spectrum of Aqn benzylTSC Cr(III) Complex.

then cooled to 45-60 ^{o}C. 20 mL of each medium was poured in a Petri dish and allowed to solidify. After solidification, Petri plates with media were spread with 1.0 mL of bacterial suspension, which was prepared in sterile distilled water. The wells were bored with cork borer and the agar plugs were removed. 100 μ^{-1} of the compound reconstituted in DMF (Dimethyl formamide) in concentrations of 1.0 mg/mL was added to the agar wells. DMF was used as a negative control. The plates were incubated at 37 ^{o}C for 24 & 48 hours and then the plates were observed for the growth of inhibition zones. The presence of clear zones around the wells indicated that the compound was active. The diameter of the zone of inhibition was calculated in millimeters. The well diameter was deducted from the zone diameter to get the actual zone of the inhibition diameter and the values have been tabulated. Results of bactericidal screening showed that the chelation tends to make the ligand act as more powerful and potent bactericidal agents, thus killing more of the bacteria than the ligand (Table 4).These metal complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism [19].

4. CONCLUSIONS

In conclusion, a new Cr(III) complex of Anthraquinone 4-benzyl 3- thiosemicarbazone

Table 4: Antibacterial zonal data of ligand and the Aqn benzylTSC Cr(III) complex.

S.No	Bacterial species		Zone diameter in millimeters(mm)		
1.	Candida albicabs	24 hrs (100 Conc.µ/disc)	05	48 hrs (100 Conc.µ/disc)	05
2.	Xanthomonas		05		06
3.	Pseudo aureginosa		06		08
4.	Lactobacillus casei		04		06
5.	Bacillus megaterium		04		06
6.	Staphylococcus aureus		04		06
7.	Escherichia coli		04		07
8.	Lactobacillus acidophilus		05		07
9.	Enterococus		04		06
10.	Streptococcus mutans		05		06

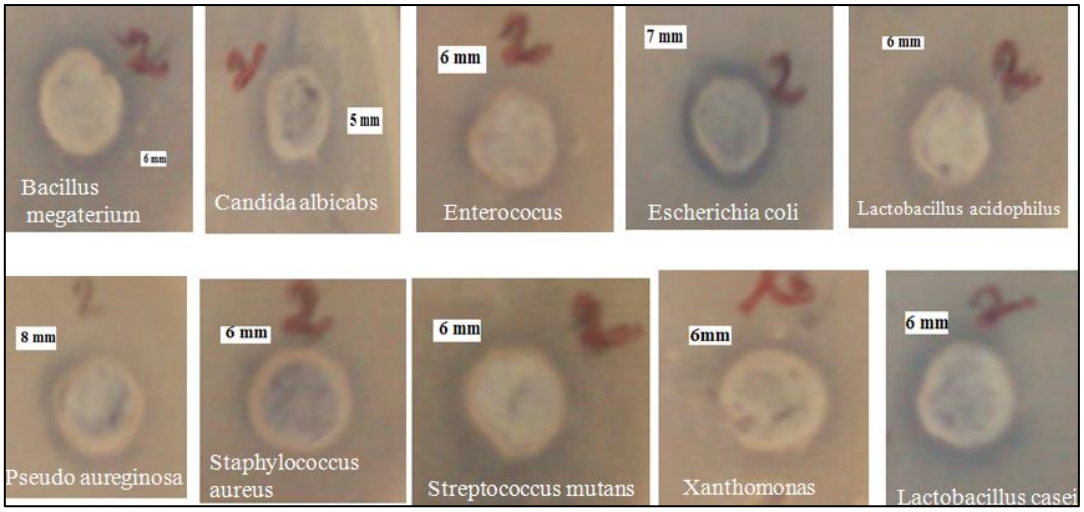


Figure 7: Bacterial Inhibition zones of 1-10 Bacteria's for Aqn benzylTSC Cr(III) Complex.

constitutes a new family of antibacterial compounds for controlling the growth of at least 1-10 bacterial species. The effect of additional binding sites, N^4 substitution and complexation with metal ion on biological activity have been given importance. Supported by the literature these compounds are expected to act as potential anticancer drugs and further studies will follow in this direction.

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****Bibliographical Sketch***

Dr. Y. Sunandamma*, Vice-principal, HOD, Associate Professor of Dept. of Chemistry, Vikrama Simhapuri University Nellore. She completed her Ph.D (1986) with DST-New Delhi academic award. She worked as an Assistant Professor, Director for Centre for Women's studies at Acharya Nagarjuna University and also Nominated to participate at NALSAR University for Women Administrators in Higher Education. She is working on UGC Major Research Project and plays a active role in Research on Inorganic ion exchange materials. Doped amino acid crystals and also Organo metallic complexes. She has 18 Publications and 8 Invited talks towards the University colleges.*