



Biochemical Behavior of Some Newly Synthesized Poly Nuclear Complexes of Tungsten

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Abstract:

Tungsten is of interest due to the role in Biological system. DithioCarbamates, the derivative of Dithiocarbamates acids are important on account of their wide applications; it contains Sulphur donor atoms which have tendency to form complexes with Tungsten Metal. Polynuclear complexes of Tungsten have been Synthesized and characterized by different methods. Physical and Analytical measurements like conductivity measurement, TLC Technique were used to check the purity of Synthesized compounds. Physico-chemical methods like IR, Electronic, ^1H NMR spectral studies have been used to find out the geometry of Synthesized complexes. Result shows that dithiocarbamates ion allows the formation of strong multinuclear bridging complexes with Tungsten metal with reduced tendency to dative π bond formation from metal to sulphur atom.

Biocidal studies of compounds against certain microbial species have been carried out by serial dilution method using BOD incubator to evaluate the MIC (Minimum inhibitory concentration) value. On the basis of MIC value it is interpreted that Tungsten complexes are most active as compared to ligand fragments. Plasmodium species were used for antimalarial screening. Antimalarial activity is expressed in IC_{50} value. In addition The DNA binding ability of the active compounds was examined by using biophysical spectroscopic studies. These studies indicate that the compounds can intercalate and stabilize DNA. This work has great importance in the field of Bioinorganic relevance because there is a major relationship between the Physiological role of the enzyme formate dehydrogenase.

Key Words:

Antimalarial, Biocidal, DNA Binding affinity, IC_{50} value, Spectra, Synthesis.

Introduction:

The dithiocarbamates ^[1] were discovered as a class of chemical compounds early in the history of organosulphur chemistry. The parent compound, dithiocarbamic acid can be obtained as colorless,

needles from its ammonium or sodium salts by treatment with cold acid. The dithiocarbamate ligand is one of those polyatomic ligands which can stabilize higher oxidation states of Transition metals in their complexes. The strong metal binding properties of dithiocarbamates are directly related with the presence of two donor sulphur atoms which primarily determine the nature of the metal that can be bound and the strength of complexes formed. Multinuclear compounds of Tungsten have been synthesized and characterized by different methods. The compounds of Tungsten have biological properties ^[2] which include antimicrobial, antimalarial and anticancer activities. Moreover drugs that are capable of targeting DNA are of substantial interest because their potential use in cancer chemotherapy. Interaction between small molecules and DNA has emerged as an area of research being widely investigated. These interactions play a central role to define the local structure of Nucleic acids as well as to design novel drug. chemotherapeutic agents etc. Metal complexes can bind to DNA through a series of interactions like coordination by the DNA bases, intercalation and Non-covalent interaction including hydrogen bonding between the coordinated ligands and phosphate oxygen atoms of the sugar phosphate group. Binding may induce cleavage of Nucleic acids which may be considered as an enzymatic reaction comprising various biological processes as well as biotechnological manipulation of genetic material.

The synthesized tungsten complexes were studied for Biocidal effect, antimalarial activity and as well as the effect of metal ion on the DNA binding mode. This work has great importance in the field of Bioinorganic relevance.

Materials and Methods

All chemicals and reagents were of reagent grade solvents and chemicals were purified and dried by Standard methods. The ligands and tetrathiometalate of Tungsten were prepared by the reported procedures, FTIR were recorded on Thermo Nicolet Avatar 370. Electronic spectra on Varian Cary 2390 UV-Visible-NIR Spectrophotometer, NMR Spectra on Bruker Avance III, 400 MHz Spectrometer.

The Conductance measurements were carried out in a Toshniwal CL-01-06 Conductivity Bridge using 1×10^{-3} M DMF solutions. CD measurements were recorded on a JASCO (J-810) Spectro-polarimeter keeping the concentration of DNA constant (5×10^{-5} M) while varying the complex concentration ($r_i = [\text{complex}] / [\text{DNA}] = r_i = 0, 0.4, 0.5$).

Synthesis of Complexes:

0.001 mol ammonium Tetrathio tungstate solution in 15.0 mL water was added to solution of 0.001 mol diethyldithio-Carbamate/ethyenebisdithio carbamate/Ammonium Phenylthio-carbamate in minimum Quantity of double distilled water. The mixture solution was well stirred with the help of magnetic stirrer and it was reduced to one third of its original volume on boiling and filtered hot. Now solution was kept into refrigerator for overnight. A coloured crystalline product was obtained which was washed with water, ethanol followed by ether and finally dried in Vacuo.

All the compounds were tested for their Biocidal activity by using serial dilution method^[3, 4] against Bacterial species viz. *Escherichia coli* (gram-ve) and *Staphylococcus aureus* (gram+ve) (Incubation period for Bacteria 24 hr at 37°C) Fungal species viz. *Aspergillus flavus* and *Helimentosporium Ozyae* (Incubation period for Fungi 96 hr at 28°C) Using BOD incubator all operation must be conducted in aseptic experimental condition in order to avoid the atmospheric external contamination.

In vitro antimalarial activity

Plasmodium falciparum species were grown, adapted and maintained in vitro routinely. The *P. falciparum* culture was Synchronized to ring stage and diluted with fresh human erythrocytes. The invitro assay of inhibition of schizont maturation was followed for screening the antimalarial activity of the compounds. The experiment was done in 24 well microtitre plates in the presence of various concentrations of drugs followed by standard method^[5, 6]. The control wells were without drug. The growth was monitored after 24-36 h of culture when the parasites would have developed to schizont stage. Percent inhibition was calculated according to the following formula: % inhibition = $100 \left(\frac{\text{No of schizonts or 200 asexual parasites in test well}}{\text{No of schizonts or 200 asexual parasites well}} \right) \times 100$.

In vivo Antimalarial Screening (Blood scheizontocidal activity)

Healthy swiss albino mice (4-6 week old) and *P. berghei*, sensitive to chloroquine. were used in the

study. The animals were divided into groups consisting of 5 mice each. All the groups were injected *P. berghei* infected red blood cells in volume of 0.1 mL (diluted in phosphate buffer (PBS) on day 0 intraperitoneally. All the experimental groups received the drugs in different concentration on day 0 to day 3. Control group received only PBS and one group was given chloroquine (IC_{50} value = 0.03 mg/mL) 3mg/kg as a standard antimalarial drug. On day 4 thin blood smears were prepared from the tail vein to monitor the parasitaemia. Percent inhibition of the parasitaemia in calculated against the control group. The chloroquine recipients group was negative throughout. Many techniques have been applied for investigation of the interaction of metal complexes with DNA these include UV-Visible studies, CD spectral studies.

Results and Discussion:

All the complexes are non hygroscopic and stable at room temp they are insoluble in water and common organic solvents but soluble in DMF and DMSO. Compounds were routinely checked for their purity on Silica gel-G TLC plates and spots were visualised by iodine vapours. Molar conductance values of the complexes were found to be less than $30 \square 1 \text{ cm}^2 \text{ Mol}^{-1}$ suggesting non-electrolytic nature of the complexes.

Ir spectra:

The IR spectra of all the dithiocarbamate fragments exhibited sharp and prominent bands at $1500-1350 \text{ cm}^{-1}$, $944-900 \text{ cm}^{-1}$, $760-740 \text{ cm}^{-1}$ and $1590-1540 \text{ cm}^{-1}$ these were assigned to the presence of ν (C-N), ν (C-S-S asy), ν (C-S-S Sym.) and 2 (N-H) bending groups respectively. In the IR spectra of metal complexes there is no coordination through Nitrogen atom because there is almost no shifting of the band position of Nitrogen centres and during complexation the bands which all exhibited by ligands are shifted towards lower frequency region.

A strong bands around the regions $500-494 \text{ cm}^{-1}$ and $444-449 \text{ cm}^{-1}$ suggesting the presence of ν (W-S) t and ν (W-S) b bands respectively the medium intensity stretching vibrations at region $383-343 \text{ cm}^{-1}$ confirms the two metal centres in the binuclear type of complexes are linked to each other through the two bridging sulphur atoms showing the W-S-W bands.

Electronic Spectra :

Dithiocarbamates show high intensity absorption in the ultraviolet region. The water soluble alkali metal or ammonium salts of dithiocarbamic acid display two bands one in the region of 40000 cm^{-1} - 38461 cm^{-1}

which are associated with polarization of nitrogen conjugation. The other slightly, more intense at about 34482cm^{-1} - 33898cm^{-1} is associated with sulphur conjugation. In the U.V. region an intense band appears at 38461cm^{-1} due to intra ligand π - π^* transition of the N=C=S groups. The intra ligand π - π^* transition of the S=C=S group, generally appears as a shoulder band associated with non-equivalence of the C=S bands of the ligand. These bands tend to disappear in the case of metal complexes, showing thereby that the dithiocarbamate ligand S-S bonded to the metal ions. Bridging complexes of Tungsten are highly colored due to deep colouration, their electronic spectra do not display well-resolved bands. In high energy region, the complexes display two or three bands associated with ligand molecules. The strong band at about 23000cm^{-1} - 25800cm^{-1} in the complexes are assigned to charge transfer (transition of sulphur to tungsten (S \rightarrow W)). The complexes display d-d transitions bands in the range 14788 - 13346cm^{-1} and $12,600$ - 12475cm^{-1} respectively. These bands are attributed to $3E \rightarrow 1A^1$, and $1E \rightarrow 1A^1$, transitions which indicate the presence of dithiocarbamate bound to tungsten (V).

^1H NMR Spectra:

^1H nmr spectra of the tungsten complexes Nos. 2a, 3a & 4a in (DMSO- d_6 ; TMS) showed the signals at $\delta 1.2$ (12H, t CH_3), $\delta 4.1$ (8H, q, CH_2), $\delta 9.5$ (4H, S,NH), $\delta 3.8$ (8H, br S, CH_2) $\delta 7.09$ - 7.94 (20H, m, C_6H_5) and $\delta 10.8$ (2H, S, NH) respectively.

A singlet observed at $\delta 3.10$ - 3.42 (ppm) in the parent dithioacid and assigned for SH proton is found to be absent in the spectra of corresponding tungsten complexes indicating the deprotonation of SH group and formation of W-S band. This fact is also in agreement with IR and Electronic spectral studies.

Biocidal Studies:

All the complexes were tested for their Biocidal activity by using serial dilution method against bacteria and Fungi. The observed MIC values of the compounds have been presented in table 1. The compound No. 1 which is used as starting material for the synthesis of tungsten is biologically in active against both the bacteria and fungi. This is probably either due to lipid insoluble nature (Water soluble character) or their instability in the solution. The compounds (Nos. 2,3,4) which are Quite antibacterial and antifungal in nature as indicated by their MIC values but their Biocidal activity is retarded in their synthesized complexes due to their non availability in free state to the cellular fluids of microbial cells and thus their diffusion through the cell membrane is considerably inhibited causing only their small concentrations in the values. The compounds (Nos. 2a, 3a, 4a) are the binuclear sulphur rich compounds of tungsten since these are fairly stable and water insoluble they can easily pass through the cytoplasmic membrane and become lipid soluble inside the cell causing bacteriostatic and fungitoxic effects.

Table-1: Minimum Inhibitory Concentration (MIC) values in Molar Concentration ($\times 10^{-4}$) of the ligands and complexes

Compound	No.	Bacteria		Fungi	
		E. Coli	S. Aureus	A. Flavus	H. ozyzae
$(\text{NH}_4)_2 \text{WS}_4$	1	2.8735	2.1551	1.4367	1.4367
$\text{C}_5\text{H}_{10}\text{NS}_2\text{Na}$	2	0.3654	0.3654	0.1827	0.7309
$\text{C}_4\text{H}_6\text{N}_2\text{S}_4\text{Na}_2$	3	0.1220	0.0610	0.0610	0.0610
$\text{C}_7\text{H}_{10}\text{N}_2\text{S}_2$	4	0.6720	0.6720	0.3360	0.1677
$\text{W}_2[\text{C}_{10}\text{H}_{20}\text{N}_2\text{S}_8]$	2a	0.0197	0.0197	0.0394	0.0395
$\text{W}_2[\text{C}_8\text{H}_{12}\text{N}_4\text{S}_{12}]$	3a	0.0085	0.0042	0.0170	0.0170
$\text{W}_2[\text{C}_{12}\text{H}_{12}\text{N}_2\text{S}_8]$	4a	0.0375	0.0046	0.0093	0.0187

Antimalarial activity:

Results of antimalarial activity are mentioned in Table (2) compounds 3a, 4a have been found to show significant antimalarial activity than the chloroquine. Other compounds showed mild to moderate activity it may further conclude that Introduction of high percent of sulphur content in the compounds increase to antimalarial activity.

It was also observed that antimalarial activity was slightly increased in the complexes (Nos. 2a, 3a, 4a) as compared to ligands (Nos 2,3,4) because the complex formed with the metal ion is so strong that it does not dissociate inside the cells in cellular fluid to release again free bioactive ligand. The

liposolubility of the free ligands is retarded in their complex.

Table-2: Antimalarial Activity

Compound	Sample No.	IC ₅₀ value (microgram /mL)
C ₅ H ₁₀ NS ₂ Na	2	18
C ₄ H ₆ N ₂ S ₄ Na ₂	3	12
C ₇ H ₁₀ N ₂ S ₂	4	15
W ₂ [C ₁₀ H ₂₀ N ₂ S ₈]	2a	6
W ₂ [C ₈ H ₁₂ N ₄ S ₁₂]	3a	2
W ₂ [C ₁₄ H ₁₂ N ₂ S ₈]	4a	5
Chloroquine	SD	0.03

DNA Binding –UV/Vis Studies:

Absorption spectroscopy is one of the most useful techniques to study the binding of any drug to DNA. The extent of hypochromism generally indicate the inter calation binding strength [7]. The hypochromicity characteristic of inter cation has usually been attributed to the interaction between the electronic states of compound and those of DNA bases. The hydrochromic effect might be ascribed to external contact (electrostatic) binding or to partial uncoiling of the helix structure of DNA, exposing more bases of DNA. On the basis of some spectroscopic titrations it is observed that a large bathochromic shift (18-23 nm) and a high percentage of hypochromicity of the UV-Visible soret band is an indication of ligand intercalation with DNA [8]. The extent of hypochromicity is directly proportional to the strength of ligand intercalation. Hypochromicity of the complexes soret bands was notices on incremental addition of calf thymus DNA (CT DNA). The extent of hydrochromicity of tungsten complex indicate the strong intercalative mode of interaction with DNA.

In tungsten complex the spectroscopic Titration curve (Fig.1) shows two isobestic points at 235 nm and 280 nm respectively. This indicates that there is an equilibrium state between metal complex with CT DNA & the interaction of W (V) diethiocarbamate complex with DNA takes place in multiple steps.

Circular Dichroism Spectroscopy:

Circular dichroism (CD) studies on DNA and DNA-ligand complexes individually indicate the changes in the DNA structure and conformation upon ligand interaction. CD spectra of CT-DNA were recorded at increasing complex/CT-DNA ratios. The observed CD spectrum of natural calf Thymus DNA consists of a positive band at 275 nm due to base stacking and a negative band at 245 nm due to helicity which is characteristic of DNA in right handed B form [9,10] (Fig.2). The effect of Tungsten complex on the conformation of the secondary structure of DNA was studied by keeping the concentration of CT-DNA at 5×10⁻⁵M while varying the concentration of complex in a buffer solution of 10mM of Tris (ri=0,0.4, 0.5). The spectrum of CT-DNA and those with the additives were monitored from 215-800 nm. In the presence of tungsten complex, the intensity of the positive peak increased and the intensity of the negative peak decreased. The changes in the CD spectra in the presence of complex show stablization of the right handed B form of CT-DNA.

Figure 1: UV-Visible spectra of DNA ($5 \times 10^{-5} \text{M}$) in the absence and presence of increasing amounts of tungsten complex ($r_i = [\text{DNA}]/[\text{complex}]$)

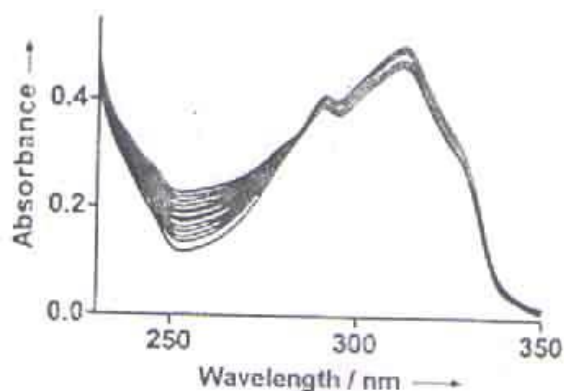
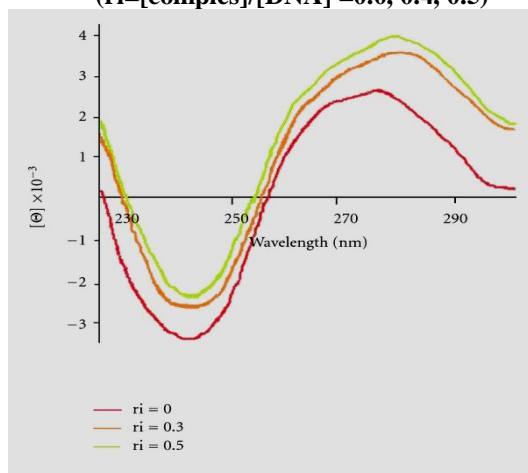


Figure 2: CD-Spectra of CT-DNA ($5 \times 10^{-5} \text{M}$) in the presence of increasing amounts of tungsten complex at the following. Stoichiometric ratios ($r_i = [\text{complex}]/[\text{DNA}] = 0.0, 0.4, 0.5$)



Conclusion:

In this paper we investigate, synthesis, characterization of new tungsten complexes with dithiocarbamate ligands, Biocidal, antimalarial activity and the DNA binding ability of the complex was examined by using biophysical spectroscopic studies. The invitro antimicrobial studies revealed that the tungsten complexes are potentially active against Bacterial and fungal species and exhibit greater Biocidal effect as compared to the ligand fragments. The compounds were found to exhibit significant antimalarial activity the results suggest that antimalarial activities are strongly affected by percentage of sulphur content. The DNA binding ability of complex indicates that the complex can

intercalate and stabilize DNA. Our finding may give a new application and open a new way in the design of more effective and useful tungsten complexes for synthetic as well as medicinal chemists.

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