Syhthesis, Characterization and Antimicrobial Studies of Isatin Thiosemicarbazones Derivatives of Dichlorobis(cyclopentadienyl) hafnium(IV)

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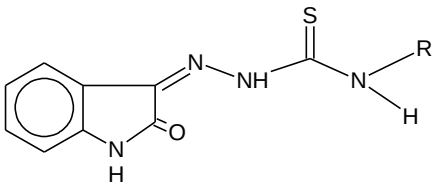
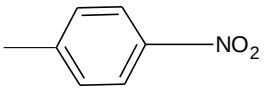
ABSTRACT---- ***The reactions of Cp_2HfCl_2, with thiosemicarbazones (LH) derived from isatin and 4-nitrophenyl substituted (IPTSH) and 4-methyl phenyl substituted (IMTSH) thiosemicarbazide in various stoichiometric rations have been studied and the complexes of the type $[Cp_2HfCl(L)]$ and $[Cp_2Hf(L)_3]$ are obtained. Tentative structural conclusions are drawn for the reaction products based upon elemental analyses, electrical conductance, magnetic moment and (UV-Vis, IR, ^1H NMR and ^{13}C NMR) spectral data. Gram positive, gram negative bacteria and unicellular, multicellular fungi have been used for the evaluation of antimicrobial activity with selected antibiotics for the comparison. 4-Nitro Phenyl substituted moiety exhibited promising antibacterial and antifungal activities.***

Keywords--- Cp_2HfCl_2, Thiosemicarbazones, IPTSH, IMTSH, Antimicrobial activity

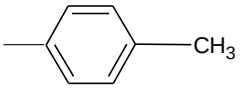
1. INTRODUCTION

The literature survey indicates that the isatin derivatives are known to be associated with broad spectrum of biological activity like antibacterial (1), anti-inflammatory (2), analgesic (3), anti-viral (4), anti-fungal (5) and anti-tubercular (6). Though biological activity of heterocyclic thiosemicarbazones have been studied in detail but practically no study has been done on their coordination behavior. Before one decade, Bain G.A. *et al*, 1997 (7) published a paper on Copper (II) complexes with N-4-substituted isatin thiosemicarbazone. It is evident from literature that no work has been done on hafnium (IV) heterocyclic thiosemicarbazones complexes. So it has been considered of interest to investigate the synthetic and structural aspects of the complexes of dichloro bis(cyclopentadienyl) hafnium(IV) with isatin thiosemicarbazones (I). The structure of ligands are shown below in **Fig. (I).**



Where, R= 

(IPTSH)



(IMTSH)

(I)

2. EXPERIMENTAL

All operations were performed under strictly anhydrous conditions. Extreme precautions were taken to exclude moisture. Tetrahydrofuran was dried and stored over Na wire and then boiled under reflux until it gave the characteristic blue colour with benzophenone. N-Butylamine and triethylamine were dried by standard method (8). Cp_2HfCl_2 was purchased from Aldrich Chemical Co. The ligands were prepared as according to Dongli C. *et al*, 1994 (9). The details of analysis and physical measurements were the same as literature (10,11).

2.1. Preparation of Complexes

2.1.1. $Cp_2HfCl(L)$

To a solution of bis(cyclopentadienyl) hafnium (IV) dichloride (10 mmol) in dry tetrahydrofuran (ca. 50 cm^3) was added thiosemicarbazone (10 mmol). A clear solution was obtained. To this triethylamine (10 mmol) was added. The mixture was stirred for 35-40 hrs. The triethylamine hydrochloride was precipitated out, which was removed by filtration. The solution was filtered and its volume was reduced to 15 cm^3. To this petroleum ether (b.p. 60-80^0) (15 cm^3) was added and solution was allowed to stand overnight. The coloured crystals so obtained, were filtered, thoroughly washed with ether and dried in vacuo at room temperature.

2.1.2. $Cp_2Hf(L)_3$

Bis(cyclopentadienyl) hafnium (IV) dichloride (10 mmol) was dissolved in anhydrous tetrahydrofyran (50 ml) and then thiosemicarbazone (30 mmol) was added. To this solution, n-butylamine (10 mmol) was added. The mixture was stirred for 35 hrs. The precipitated complex was removed by filtration and thoroughly washed with tetrahydrofuran and dried under vacuo at room temperature.

Details of the reactions and the analytical data of the products are given in **Table 1.**

2.2. Antifungal and Antibacterial Activities

Bacterial and fungal growth inhibition was performed by Disc diffusion method and for bacteria nutrient agar and bor fungi Sabouraud Dextrose Agar medium have been used. Amikacin and Griseofulvin were used as reference standards for bacteria and fungi respectively. Staphylococcus aureus (gram positive), Streptococci (gram positive), Klebsiella pneumoniae (gram negative) and E.coli (gram negative) bacteria whereas, Aspergillus Niger (Unicellular) and Candida albicans (Multicellular) fungi have been evaluated for the present study. Test compounds and standards were dissolved in DMSO (Dimethyl Sulfoxide). The activity was checked at two concentrations, 100 µg/ ml and 10 µg/ml. Solution was applied by micropipette on the filter paper disks, placed on the agar plate. The surface of the plate was inoculated with bacteria and fungi. Incubation was done for 24 hours at 37^{0}c. The diameter of the zone of inhibition was measured at the end of the incubation period and MIC (Minimum inhibitory concentration) and MBC (Minimum bacterial Concentration) have been calculated and tabulated. Quality parameters are important to be ensured in the use of combi-discs to ensure that correct and reproducible results are obtained. For quality control of disc diffusion, NCCLS has recommended the

following ATCC reference strains namely *E. coli*. ATCC 25922, *S. aureus* ATCC 25923, *Streptococci* ATCC 49619, Klebsiella ATCC 700603, Aspergillus niger ATCC 9029, Candida albicans ATCC 2091. When these strains produce the results that fail within the specified limits (as per NCCLS guidelines), the susceptibility test results are considered to be valid (12,13).

3. RESULT AND DISCUSSION

The reactions of bis(cyclopentadienyl) hafnium (IV) dichloride, with isatin thiosemicarbazones (LH), in different molar ratio, in dry THF in the presence of triethylamine or n-butylamine, are represented by the following general equations :

$$Cp_2HfCl_2 + LH + Et_3N \xrightarrow{THF} [Cp_2HfCl(L)] + Et_3N.HCl$$

$$Cp_2HfCl_2 + 3LH + 2n\text{-}BuNH_2 \xrightarrow{THF} [Cp_2Hf(L)_3] + 2n\text{-}BuNH_2.HCl + C_5H_6$$

The physical properties and analytical data of the complexes are given in **Table 1**. The complexes of the type $[Cp_2HfCl(L)]$ are soluble in THF, DMF, DMSO, pyridine and nitrobenzene.

The complexes of type $[CpHf(L)_3]$ are partially soluble in DMF and DMSO. The electrical conductance measurements show that non-electrolytic nature of the complexes. All these complexes are diamagnetic in nature.

3.1. Electronic Spectra :

The electronic spectra of all these complexes show a band in the region 22800-23400 cm^{-1}, which can be assigned to the charge transfer band (14). In addition, the ligands and complexes show bands at ca. 32800-34200 cm^{-1}, which may be assigned to the intra-ligand transitions.

3.2. Infrared Spectra :

The infrared spectra of the complees show bands at ca. 3000 cm^{-1}, 1430 cm^{-1}, 1000 cm^{-1} and 810 cm^{-1} indicating the presence of cyclopentadienyl ring attached hafnium (IV) ion. All these bands are similar to those reported (15) for bis (cyclopentadienyl) hafnium(IV) chloride. The appearances of these bands for cyclopentadienyl ring indicate that (η^5-C_5H_5) group remains in the complexes.

The infrared spectra of ligands show bands at ca. 3320, 3230 and 1600 cm^{-1} assignable to $\upsilon(N^4H)$, $\upsilon(N^2H)$ and υ(C=N), respectively. In the complexes the first band remains almost at the same position, indicating the non-coordination of hydrazinic nitrogen atom to hafnium. The complex show the absence of band at 1600 cm^{-1} to lower frequency (ca 15-20 cm^{-1}) indicating the coordination of azomethine nitrogen to hafnium. In the far-infrared spectra of the complexes, the bands appearing at ca. 460-475 cm^{-1} are tentatively assigned (16) to (Hf-N) vibration.

The four bands occurring in the regions, 1460-1500 cm^{-1}, 1270-1280 cm^{-1}, 1040-1020 cm^{-1} and 785-760 cm^{-1} may be assigned (17,18) to thioamide vibrations. The appearances of these four bands indicate the existence of the ligands in the thione form in the solid state. These bands of the ligands due to mixed contributions of δ(N-N), υ(C-N), υ(C-S) and δ(C-H) vibrations, are found to be absent in the spectra of complexes indicate the possibility of thione $\rightleftharpoons$ thiol tautomerism. The infrared spectra of complexes show a new band at ca 580-600 cm^{-1} which is due to (19) conversion of C=S to C-S^-. The appearance of a new band in the complexes at ca. 340-360 cm^{-1} is assigned to (Hf-S), and show that the sulphur is bonded to hafnium atom. The spectra of the ligands show vibration at ca. 3180 cm^{-1} and 1680 cm^{-1}, which are assigned (20) to υ(N-H) and υ(C=O) vibrations of isatiin moiety. These bands persist in the complexes indicating the non-involvement of these groups in bond form atom with hafnium.

3.3. Proton Magnetic Resonance Spectra

The proton magnetic resonance spectra of the complexes $[Cp_2Hf(L)Cl]$ were recorded in deuterated dimethylsulphoxide (**Table 2**). The spectra of complexes of the type $[CpHf(L)_3]$ could not be taken due to their poor solubility. The

intensities of all the resonance lines were determined by planimetric integration. A comparison of the spectra of the ligands and those of the complexes leads to the following conclusions:

(a) A singlet in all derivatives at δ6.54-6.80 may be assigned to the proton of cyclopentadienyl ring and indicate the rapid rotation of the ring about the metal ring axis.

(b) The proton signals of N(2)H and N(4)H are seen at ca. δ9.4(1H) and δ8.5(1H) respectively. The spectra of complexes show the absence of first peak and the presence of second peak almost at the same position.

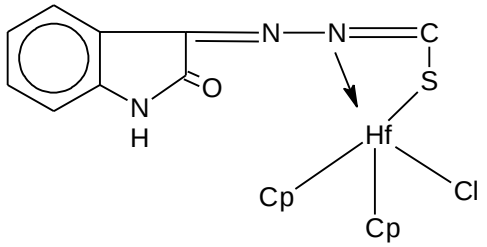
(c) The chemical shift at ca. δ7.30-7.80 ppm may be due to aromatic ring proton which also shifts downfield in the complexes. This may be due to the decrease of electron density after forming the complex.

(d) The peak due to N(1)H of isatin ring appear at ca. 11.2 ppm in the spectra of ligands and their corresponding complexes.

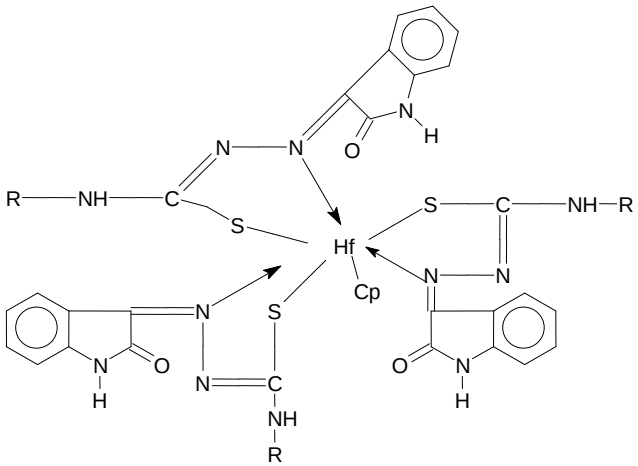
3.4. ^{13}C NMR

The ^{13}C NMR spectra of complexes were recorded in DMSO-d_6. The salient features are the cyclopentadienyl peak at δ118 (relative to TMS); the considerable shift in the position of carbon (1) (ca. δ160.0 ligands) and carbon (8) (ca. δ140.0 ligands) indicating coordination through azomethine nitrogen and thiol group. The unsubstituted. Cp ring in the ferrocenyl group show one peak (ca. δ118.0) while the substituted Cp ring shows three peaks (ca. δ160.0, 68.8, 67.5). In the complexes, these peaks remain almost at the same position as in the corresponding ligands.

On the basis of elemental analyses, electrical conductance measurements and spectral data, the following structure are tentatively proposed for $[(C_5H_5)_2HfCl(L)]$ (**Fig. II**) and $[(C_5H_5)Hf(L)_3]$ (**Fig. III**) complexes.



(II)



(III)

3.5. Antimicrobial Study

Antibacterial and antifungal activities have been performed for the both synthesized compounds and IPTSH have been found more potent against IMTSH. IPTSH exhibited inhibited the growth of *E. coli (MIC* 126.0±1.20 µg/ml, MBC 259.0±4.21 µg/ml and 20.3±0.1 mm, zone of inhibition), *Klebsiella (MIC* 441.25±3.45 µg/ml, MBC 100.33±2.22 and

17.2±0.22 mm, zone of inhibition), *Streptococci (MIC* 252.33±2.21 µg/ml, MBC 625.01±2.19 µg/ml and 16.2±0.44 mm, zone of inhibition) and *Aspergillus niger (MIC* 289.00±5.22 µg/ml, MBC 452.01±2.36 µg/ml and 13.4±0.1 mm, zone of inhibition) very efficiently.

Whereas IMTSH showed the activity against *Aspergillus niger (MIC* 398.25±16.7 µg/ml, MBC 805.23±27.7 µg/ml and 03.0±0.3 mm, zone of inhibition), *Staph aereus (MIC* 500±24.4 µg/ml and 10.1±0.3 mm, zone of inhibition), *Klebsiella (MIC* 800±89.8 µg/ml and 09.3±0.1 mm, zone of inhibition), E. *coli (MIC* 853±23.3 µg/ml and 12.3±0.1 mm, zone of inhibition), and *Streptococci (MIC* 750.0±58.8 µg/ml and 07.0±0.2 mm, zone of inhibition).

All the data of Zone of inhibition and MIC and MBC has been tabulated in **Table No. 3A, 3B** and **4** respectively.

From the above studies it can be stated that IPTSH is more potent candidature than IMTSH for the evaluation of Antimicrobial activities against bacteria and fungi and among thiosemicarbazones compound having 4-nitro phenol substitution are more active than the compound having 4-methyl phenyl group.

4. ACKNOWLEDGEMENT

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Table 1 : Characteristic data for bis(cyclopentadienyl) hafnium (IV) dichloride complexes

Reactants Cp_2HfCl_2 plus	Molar Ratio	Stirring time (hrs)	Product, colour, yield(%)	Found (calcd) %			
				C	H	N	Cl
IPTSH + Et_3N	1 : 1 : 1	40	$[Cp_2HfCl(IPTS)]$ brown, 62	43.7 (43.8)	2.9 (2.9)	10.2 (10.2)	5.1 (5.2)
IPTSH + n-$BuNH_2$	1 : 3 : 1	32	$[CpHf(IPTS)_3]$ yellowish brown, 55	47.3 (47.5)	2.5 (2.8)	16.5 (16.6)	- -
IMTSH + Et_3N	1 : 1 : 1	42	$[Cp_2HfCl(IMTS)]$ brown, 65	47.6 (47.8)	3.4 (3.5)	8.4 (8.6)	5.4 (5.5)
IMTSH + n-$BuNH_2$	1 : 3 : 1	35	$[CpHf(IMTS)_3]$ chocolate brown, 57	54.2 (54.3)	3.6 (3.8)	14.2 (14.2)	- -

Table 2: ^{1}H-Chemical shifts (δ, ppm) at 25^0C

Complex	η^5-C_5H_5	CH_3	Aromatic ring	N(4)H	N(1)H
$Cp_2HfCl(IPTS)$	6.80s	-	7.80s,8.15s	8.90s	11.28
$Cp_2HfCl(IMTS)$	6.65s	2.25s	7.82s,8.25s	8.98s	11.18

Table 3A: Antibacterial activity of IPTSH and IMTSH

Compound	Zone of Inhibition (mm)			
	Staph Aereus	Streptococci	E.Coli	Klebsiella
IPTSH	14.2±0.1	16.2±0.44	20.3±0.1	17.2±0.22
IMTSH	10.1±0.3	07.0±0.2	06.0±0.33	09.3±0.1
Standard Drug (Amikacin)	16.4±0.5	19.0±0.41	14.2±0.142	28.3±0.09

Table 3B: Antifungal Activity of IPTSH and IMTSH

Compound	**Zone of Inhibition (mm)**	
	A.niger	**Candida albicans**
IPTSH	13.4±0.1	16.5±0.44
IMTSH	03.0±0.3	05.1±0.2
Standard Drug (Griseofulvin)	16.4±0.5	19.0±0.41

Table 4: MIC and MBC Study of IPTSH and IMTSH

ORGANISM	**MIC(µg/ml)**		**MBC(µg/ml)**	
	IMTSH	**IPTSH**	**IMTSH**	**IPTSH**
Staph. Aereus ATCC 25923	500±24.4	312.33±1.44	>1000	750±66.5
Streptococci ATCC 49619	750.0±58.8	252.33±2.21	>1000	625.01±2.19
E.coli ATCC 25922	853±23.3	126.0±1.20	>1000	259.0±4.21
Klebsiella ATCC 700603	800±89.8	441.25±3.45	>1000	100.33±2.22
Candida albicans ATCC 2091	>1000	859.31±3.22	>1000	>1000
Aspergillus niger ATCC 9029	398.25±16.7	289.00±5.22	805.23±27.7	452.01±2.36

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