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Synthesis, physicoanalytical characterization and biological activity of Isatin Thiosemicarbazones derivatives of Dichloro bis (cyclopentadienyl) hafnium(IV) (Cp_2HfCl_2).

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ABSTRACT

Metal complexes were prepared by the reactions of thiosemicarbazone bearing Isatin with dichloro bis (cyclopentadienyl) hafnium (IV) (Cp_2HfCl_2) and 4-chlorophenyl substituted (ICTSH) and 4-α-naphthyl substituted (INTSH) thiosemicarbazide in various stoichiometric rations. As a result of this reaction the complexes of the type $[Cp_2HfCl(L)]$ and $[Cp_2Hf(L)_3]$ are obtained. The tentative structural identities of these synthesized compounds were elucidated by IR, ^1H NMR, ^{13}C NMR and UV-Visible spectroscopic studies. The disc diffusion method was employed to evaluate the antibacterial and antifungal activity by using Amikacin and Griseofulvin as standard drug respectively, followed by the determination of MIC and MBC by using Chloramphenicol as reference drug. The result proved that the compound ICTSH had better inhibitory activity against microbes.

Keywords: Thiosemicarbazone, ICTSH, INTSH, Antibacterial and Antifungal Activity.

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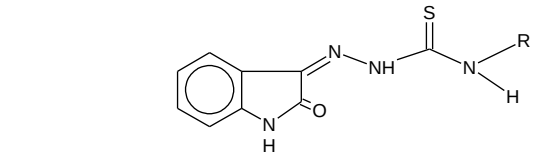
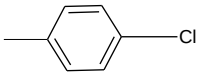
INTRODUCTION

In organic synthesis, Isatin is an important versatile lead molecule and it is synthetically important in heterocyclic chemistry. Chemically Isatin is 2,3 dioxoindole, which was obtained by the oxidation of Indigo by nitric acid and chromic acid.[1], [2]. Isatin was reported to possess Antimicrobial [3], Antioxidant [4] , Anti-inflammatory, Analgesic, Antipyretic [5],[6] Antiviral [7] , Anticancer activity[8] and Anti-tubercular[9] activity.

Several literatures reported the synthesis and biological evaluation of isatin thiosemicarbazone derivatives[10],[11],[12] but there are very few authenticate evidence of hafnium (IV) heterocyclic thiosemicarbazones complexes [13]. 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=  ICTSH

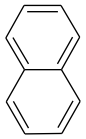
 INTSH

Fig. (I)The Structure of Ligands

EXPERIMENTAL

All reactions and techniques ware performed under anhydrous conditions. Tetrahydrofuran (THF) (JT Baker, b.p. 65-66^{0}c) is predried over KOH pellets and then it is dried by heating under reflux over sodium wire in the presence of Benzophenone, as indicator, until it produced Blue Colour[14]. N-Butylamine and triethylamine were dried by standard method as given in literature [15]. Cp_2HfCl_2 was purchased from Aldrich Chemical Co. The ligands were prepared as reported earlier in literature[16]. The details of analysis and physical measurements were the same as in different literature [17],[18].

Preparation of $Cp_2HfCl(L)$ from the reaction of Cp_2HfCl_2 with Triethylamine (Et_3N)

10 mmoL Thiosemicarbazone was added to a 10 mmoL solution of bis (cyclopentadienyl) hafnium (IV) dichloride in dry tetrahydrofuran (ca. 50 cm^3) and a clear solution was obtained. Thereafter, Triethylamine (10 mmol) was added to the above solution. The solution mixture was stirred for 35-40 hrs. A precipitate of triethylamine hydrochloride was formed which was removed by filtration and the final volume of the solution was reduced to 15 cm^3. Petroleum ether (15 cm^3, b.p. 60-80^{o}c) was added to the solution and allowed to stand for overnight. As a result the coloured crystals are obtained, filtered and thoroughly washed with ether and dried in vacuum at room temperature.

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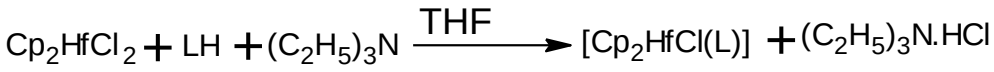
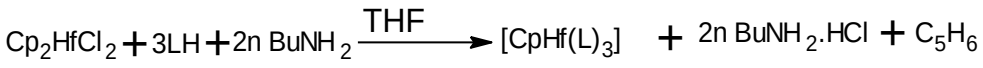


(LH= ICTSH and INTSH)

Preparation of $Cp_2HfCl(L)_3$ from the reaction of Cp_2HfCl_2 with n-butylamine (n-$BuNH_2$)

10 mmoL of Bis(cyclopentadienyl) hafnium (IV) dichloride was dissolved in 50mL of THF(anhydrous) and to it 30mmoL thiosemicarbazone was added. In this solution 10mmoL of n-$BuNH_2$ was added with continuous stirring for 35 hours. The complex which was precipitated, was removed by filtration and washed with THF and dried under vacuum at room temperature.



(LH= ICTSH and INTSH)

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

Antifungal and Antibacterial Activities

*S. aurus, E.coli,*and *Pseudomonas aeruginosa* (bacterial strain) and *Aspergillus niger, Candida albicans* (fungal strain) were chosen for the activity.

In-vitro antimicrobial activities were performed and examined by agar diffusion method and activity testing was carried out by agar cup method. Ampicillin and Griseofulvin were taken as standard drug for bacteria and fungi respectively. The activity was experimented for 10µg/mL. The activity was determined by the measurement of diameter of zone of inhibition in mm. Minimum Inhibitory Concentration (MIC) were measured and tabulated. [13].

RESULT AND DISCUSSION

The reactions of bis(cyclopentadienyl) hafnium (IV) dichloride and isatin thiosemicarbazones (LH), with triethylamine and n-butylamine from which $[Cp_2HfCl(L)]$ and $[CpHf(L)_3]$ were formed respectively. The reaction was done in different molar ratio in the presence of dry THF. The details of reactions, physical properties and analytical data of these complexes are given in **Table 1**.

Table 1: Analytical Data

Reactants Cp_2HfCl_2 plus	Molar Ratio	Stirring time (hrs)	Product, colour, yield(%)	Found (calcd) %			
				C	H	N	Cl
ICTSH + Et_3N	1 : 1 : 1	40	$[Cp_2HfCl(ICTS)]$ Light brown, 68	44.3 (44.6)	2.8 (3.0)	8.2 (8.3)	10.3 (10.5)
ICTSH+n-$BuNH_2$	1 : 3 : 1	35	$[CpHf(ICTS)_3]$ brown, 58	48.6 (48.7)	2.8 (2.9)	13.5 (13.6)	8.4 (8.6)
INTSH + Et_3N	1 : 1 : 1	45	$[Cp_2HfCl(INTS)]$ Light brown, 60	50.5 (50.5)	3.2 (3.4)	8.0 (8.1)	5.1 (5.1)
INTSH + n-$BuNH_2$	1 : 3 : 1	40	$[CpHf(INTS)_3]$ Dark brown, 60	58.0 (58.1)	3.3 (3.5)	13.1 (13.1)	- -

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It was found that the complexes, [$Cp_2HfCl(L)$], were soluble in DMSO, THF, DMF, Pyridine and Nitrobenzene whereas, the solubility of [$CpHf(L)_3$] complexes were found partial in DMF and DMSO. The nature of these complexes were found as non-electrolytic and diamagnetic.

All the complexes showed a band within the region of *ca.* 22800-23400 cm^{-1} which is considered to be a band for charge transfer, along with a band of ligand and complexes in the region of *ca.*32800-34200 cm^{-1} was found which can be assigned as Intra-ligand transitions [19].

The infra-red spectra analysis of different complexes showed bands at *ca.* 3000 cm^{-1}, 1430 cm^{-1} 1030 cm^{-1} and 815 cm^{-1} which stipulated the presence of hafnium (IV) ion (η^5 C_5H_5-) adjoined with Cyclopentadienyl ring.[20] studied the same and observed the same data and thus this data supported the existence of η^5 C_5H_5 ring. The I.R. spectra of ligands found same as ($\upsilon(N^4H)$ = *ca.*3320 cm^{-1}, $\upsilon(N^2H)$ = *ca.*3230 cm^{-1} and υ(C=N) = *ca.*1600 cm^{-1}) described earlier in related literature. Hydrazinic nitrogen atom bonded non-coordinately to Hf was indicated by the band at *ca.*3320 cm^{-1}, almost at the same position of first band of ligand. There was no band for complex at *ca.*1600 cm^{-1}, for (Hf-N) vibration the bands appeared at *ca.*460-475 cm^{-1} and at *ca.*15-20 cm^{-1} for the coordination of azomethine nitrogen atom to Hf [21].

A band near *ca.*1500 cm^{-1} (*ca.*1460-1500 cm^{-1}) and *ca.*1250 cm^{-1}(*ca.*1270-1280 cm^{-1}) were found which resembles to Thioamide due to C-NH vibrations, along with a band near *ca.*700 cm^{-1} was found which was due to N-H out of plane wagging [22]. These bands confirm the existence of ligands in Thione form. But, these bands are absent in complexes, may be due to Thione-Thiol (NH-SH) Tautomerism. A new band below 600 cm^{-1} was visible which was due to conversion of C=S to C-S, several literatures supports the data[23].

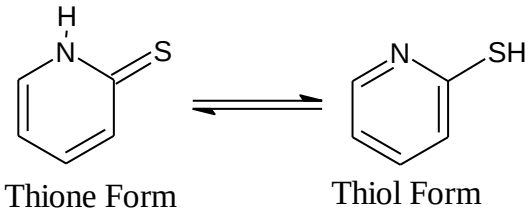

Fig. (II)Thione Form and Thiol Form

The band at *ca.* 340-360 cm^{-1} was found which indicated for the Hf-S. The spectra in the region of *c.a.* 3180, 1680, 1660 cm^{-1} resembles to Isatin derivative containing NH, C=O and C=N groups [24].

The PMR (Proton Magnetic Resonance) spectra were recorded in dimethyl sulfoxide-d_6. The comparison of PMR Spectra of the ligands and complexes showed the following characteristics. The spectra of complexes of the type [$CpHf(L)_3$] could not be taken due to their poor solubility. The chemical shift due to Isatin N(1)H ring proton was found at δ11.2 ppm and 11.25 ppm for INTSH and ICTSH respectively. A singlet at δ6.54-6.80 were assigned for the proton of C_5H_5- (Cyclopentadienyl ring), δ9.4 and δ8.95, δ8.92 signals were found for N(2)H and N(4)H respectively, and for the complex the spectrum of N(2)H are not seen. The chemical shift at δ7.85-8.40 were found for the aromatic ring proton. All the chemical shifts have been given in **Table2** in details.

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

Complex	η^5-C_5H_5	CH_3	Aromatic ring	N(4)H	N(1)H
Cp_2HfCl(ICTS)	6.54s	-	7.85s,8.20s	8.95s	11.25
Cp_2HfCl(INTS)	6.80s	-	7.92s,8.25s,8.40s	8.92s	11.20

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The ^{13}C NMR spectrums were also recorded in deuterated DMSO. The peak at δ118 of Cyclopentadienyl ring which is related to TMS and the peak at δ160 and δ140 were assigned for the carbons (C-1, C-8) of coordinate of Azomethine ring and Thiol group.

Substituted and unsubstituted Cp ring in Ferrocenyl ring shows following peaks at *ca.* δ118ppm and δ160, 68.8, 67.5 ppm respectively. In the complex the spectrums were similar to ligands.

On the basis of above discussion the tentative structures for $(C_5H_5)_2HfCl(L)$ (**Fig. III**) and $(C_5H_5)Hf(L)_3$ (**Fig. III**) were proposed for the complexes.

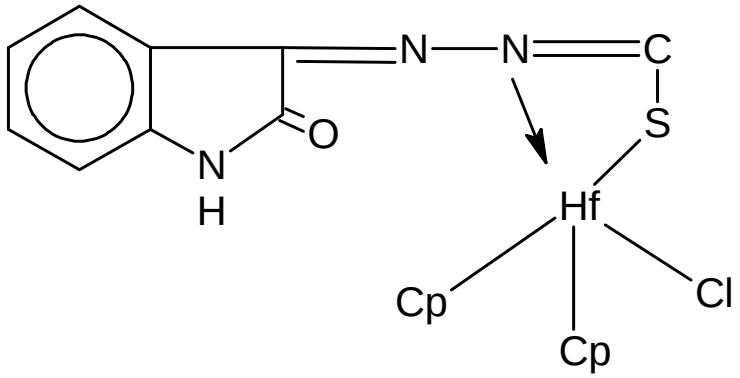

Fig. (III) Tentative Structure of Complex $(C_5H_5)_2HfCl(L)$

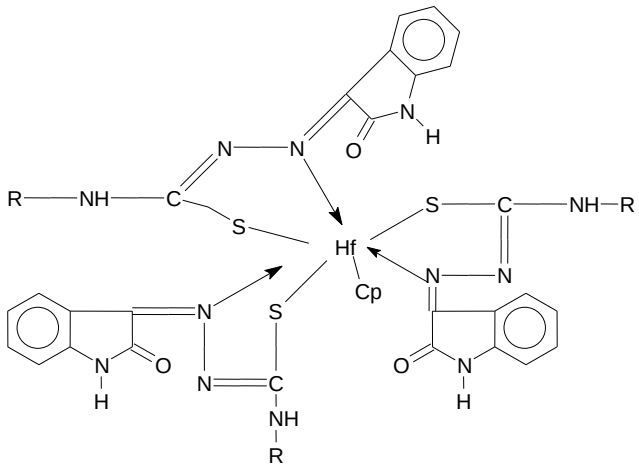

Fig. (IV) Tentative Structure of Complex $(C_5H_5)Hf(L)_3$

Antimicrobial studies were carried out for synthesized compounds against standard drugs. Previous studies supported the antimicrobial activities of hafnium nucleus which reported that zirconium and hafnium complexes show almost similar results [25]. In the present study ICTSH was found more effective candidature than INTSH. The highest activity was observed for ICTSH against all the microbes especially against *P. aeruginosa* (17.1 mm) and *Candida albicans* (17.8 mm). The details of antibacterial and antimicrobial results are given in **Table 3A,**

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3B respectively. The activity of the two complexes tested was highest against *Candida albicans*(0.005 to 0.035 mg mL^{-1}) and *P. aeruginosa* (0.020 to 0.050 mg mL^{-1}). The MIC data of both the complexes have been given in **Table 4**.

Table 3A: Antibacterial activity of ICTSH and INTSH

Compound	Zone of Inhibition (mm)		
	S. aurus	***E.Coli***	***P. aeruginosa***
ICTSH	13.2	11.2	17.1
INTSH	11.2	8.2	13.0
AMPICILLIN	18.2	14.5	21.2

Table 3B: Antifungal Activity of ICTSH and INTSH

Compound	Zone of Inhibition (mm)	
	Aspergillus niger	***Candida albicans***
ICTSH	16.4	17.8
INTSH	10.2	12.5
GRISEOFULVIN	19.5	20.2

Table 4: Minimum Inhibitory Concentration (MIC)

Compound	*MIC (mg/mL)*				
	S. aurus	***E.Coli***	***P. aeruginosa***	***A. niger***	***C. albicans***
ICTSH	0.030	0.040	0.020	0.024	0.005
INTSH	0.081	0.062	0.050	0.065	0.035

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