Synthesis and studies of ionic chelates of hafnocene with guanine

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Ionic chelates of hafnocene with guanine (I) of the type \([\eta^5-C_5H_5]_2HfL_2X\] \((\text{II})\) \([X = \text{CuCl}, \text{ZnCl}, \text{CdCl}, \text{HgCl}, \text{C}_6\text{H}_5\text{NHHCNS}_2; \text{HL} = \text{guanine})\] have been synthesized. Spectral studies (IR, UV, \(^1\)H NMR and \(^{13}\)C NMR) indicate that the guanine moiety acts as a bidentate group, binding to the hafnium(IV) ion through carbonyl oxygen at C(6) and deprotonation of N(7). Conductance measurements reveal that the compounds are 1:1 electrolytes. Fluorescence studies have been carried out for the complex containing mercury in the anionic moiety and relevant photochemical parameters have been elucidated. The thermal behaviour of the complexes has been studied by thermogravimetric and differential thermal analytical techniques. Thermodynamic parameters of the thermal decomposition process have been computed by the Coats-Redfern method and their variations have been correlated with some structural parameters of the complexes. The ligands, as well as their hafnium(IV) complexes, exhibit appreciable antibacterial and antifungal activity against \(E. coli\), \(P. aeruginosa\) and \(Z. mobilis\) bacterial strains, and \(v. anomus\) and \(A. niger\) fungal strains, respectively.

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Transition metals are essential for the normal functioning of living organisms. Therefore, it is not surprising that transition metal coordination compounds are of great interest as potential drugs. Many complexes, including the platinum group, have been synthesized and tested in a number of biological systems. With the discovery of strongly cell-mitosis depressing, broad spectrum, inorganic anticancer agent, cisplatin (i.e., cis-dichlorodiamineplatinum) by Rosenberg et al.\textsuperscript{1}, the use of metal coordination compounds in the search for new drugs for cancer treatment started to evolve and led to the discovery of interesting results\textsuperscript{5-8}. The interaction of metal ions with nucleic acids and nucleic acid constituents has been actively studied in recent years\textsuperscript{6,8}. Thus, interest in the metal complexes of guanine (2-amino-1,7-diydro-6H-purin-6-one) has arisen for the following reasons. Firstly, as a result of antitumor activity of some metal complexes, there is considerable interest in the design of model complexes involving purines which could mimic the interaction of metal ions with DNA. Secondly, growing interest in studies of metal-purine complexes relates to the bonding mode of the ligand to metal ion, as they possess divergent bonding sites. In 6-oxopurines, very frequently the exocyclic oxygen O(6) interacts with hydrogen bond donors within the metal complexes\textsuperscript{9,10}. However, coordination of 6-oxopurines by an N(7), O(6) chelation mode, as in guanine, has been frequently postulated\textsuperscript{11} and widely suggested on the basis of a number of spectroscopic and physical criteria\textsuperscript{12}.

Recent studies on determination of kinetic parameters from thermal data\textsuperscript{13,14} prompted us to analyse the variation in thermal stability of some metal complexes (II) in terms of their structural parameters. The present work is a sequel to our investigations on metal ion-biomolecule interaction\textsuperscript{15,16}.

**Materials and Methods**

Guanine (Aldrich) and hafnocene dichloride (Alfa Products) were used without further purification. Chlorine and sulphur were determined gravimetrically as silver chloride and barium sulphate, respectively. Hafnium was estimated gravimetrically as \(\text{HfO}_2\). The bactericidal activities were evaluated by cup-agar diffusion method\textsuperscript{17} while the fungi were cultured on Czapek Dox Nutrient medium\textsuperscript{18}.

Conductance measurements were made using \(1.5 \times 10^{-3} \text{M} \) solution of the complexes in nitrobenzene using Elico conductivity bridge, type CM-82. IR and UV spectra were recorded on FTIR spectrometer Spectrum 2000 and Beckman DU-64 UV-vis spectrophotometers, respectively. The \(^1\)H NMR and \(^{13}\)C NMR spectra were recorded on Bruker AC 300.
spectrometer and fluorescence spectral studies were recorded on Hitachi 650-660 spectrofluorometer. Rigaku Thermoflex, model PTC-10A, Rigaku corporation, Japan, was used for simultaneous recording of TG-DTA curves of complexes containing metal salts as anionic species, in air at a heating rate of 10° min⁻¹.

Synthesis of hafnocene complexes

\[ ([\eta^5-C_5H_5]_2HfL)_2X^- \] (II) were prepared by adding slowly, the solution of the metal salt [CuCl₂·2H₂O (0.043 g, 0.25 mmol); ZnCl₂ (0.034 g, 0.25 mmol); CdCl₂ (0.046 g, 0.25 mmol) or HgCl₂ (0.068 g, 0.25 mmol)] in acetone (10 mL) to a stirred solution of 0.095 g (0.25 mmol) of bis(\(\eta^5\)-cyclopentadienyl) hafnium(IV) dichloride and 0.038 g (0.25 mmol) of guanine (H₃L) (I) in acetone (20 mL). The contents were stirred for 8 h and filtered. Volume of the filtrate was reduced to about one-fourth of the original and petroleum ether was added. The precipitated complexes were filtered, dried and crystallized from acetone by the addition of a few drops of petroleum ether.

For the synthesis of \([([\eta^5-C_5H_5]_2HfL)_2C_6H_5NHNHC\(S\)\(_2\))^- (5), a mixture of 0.095 g (0.25 mmol) of bis(\(\eta^5\)-cyclopentadienyl) hafnium(IV) dichloride and 0.038 g (0.25 mmol) of guanine (H₃L) (I) in water (10 mL) was stirred for 8 h. This was followed by the addition of 0.055 g (0.25 mmol) of potassium phenylthiodicarbazate in a minimum quantity of water. Stirring was then continued till precipitation was complete. The product was filtered, dried and recrystallized from acetone-petroleum ether.

### Results and Discussion

The isolated complexes are pure, yellow to brown in colour and are soluble in THF, DMSO, DMF and partially soluble in water. The molar conductance measurements in nitrobenzene at \(1.5\times10^3 \text{ M}⁻¹\) for these complexes fall in the range 26.5-30.2 ohm⁻¹ cm² mol⁻¹, indicating that the complexes are 1:1 electrolytes. Analytical data of the complexes along with the decomposition temperature are given in Table 1.

The band at 1700 cm⁻¹ is attributed to 𝜽(C=O) stretching frequency in the free ligand. The guanine ligand may chelate with the metal ion through exocyclic oxygen at C(6) and deprotonation of
N(7)²²-²⁵, or only through N(7)²⁶,²⁷. The 1500-1750 cm⁻¹ portion containing the C=O, C=C and C=N stretching vibrations is where the evidence for N7/O6 chelation has generally been looked for. In the prepared metal complexes, the former band appears at ca. 1630 cm⁻¹ indicating the involvement of carbonyl oxygen at C(6) in complexation. The absorption bands at 1590 cm⁻¹ and 1675 cm⁻¹ assigned to v(C=N) and v(C=N) stretching frequencies, respectively, in free guanine shift to ca. 1570 cm⁻¹ and ca. 1650 cm⁻¹, respectively, in the metal complexes, showing that N(7) is also involved in complexation. The v(N=N-H) stretching frequency²⁸ occurs at 3435 cm⁻¹ in the free ligand, which shifts to 3445 cm⁻¹ in the complexes. Moreover, the two bands in the region 3350-3250 cm⁻¹ assigned to v(N-H) vibrational frequency in free guanine become weaker on complexation, suggesting a link through nitrogen. The v(C-S-N) stretching vibrational frequency absorbs at 1255 cm⁻¹ in both the ligand as well as the complexes. The v(C=O) stretching vibration²⁹ which absorbs at 1637 cm⁻¹ in the free guanine appears as a shoulder at 1650 cm⁻¹ in the complexes. In potassium phenyldithiocarbazate, the following IR bands are observed: the N-H stretching frequency is observed at 3400 cm⁻¹, the asymmetric CSS stretching shows a strong split band near 1000 cm⁻¹ (990, 978) indicating unidentate sulphur chelation. A strong band at 1330 cm⁻¹ is also observed which is assigned to the C-N stretching mode of the thioureide group N-C=S. The above mentioned bands remain largely unchanged in the spectrum of complex (5) indicating that phenyldithiocarbazate behaves as an anion. The band at ca. 3100 cm⁻¹ and the absorptions at 1440, 1115, 1017 and 800 cm⁻¹ are attributable to the π bonded C₆H₅ rings. In the far-IR region, bands at ca. 370 cm⁻¹ and ca. 520 cm⁻¹ in the metal complexes correspond to v(M-C) and v(M-O) stretching vibrations, respectively.

Thus, the guanine ligand acts as a bidentate group, being coordinated to the hafnium(IV) ion through carbonyl oxygen at C(6) and deprotonation of N(7).

The electronic spectrum of free guanine shows an absorption band at 285 nm (log ε 4.0) due to the π-π* transition of chromophoric carbonyl group, which, in case of the metal complexes, occurs at ca. 300 nm (log ε ca. 5.0). This shift is attributed to the involvement of C=O group in complexation. The band at ca. 245 nm (log ε ca. 3.9) observed in the spectra of both guanine and the metal complexes, has been attributed to the π-π* absorption of the C=N chromophoric group. However, the intensity of this band is higher in case of the metal complexes (log ε ca. 4.5) than the free ligand (log ε 3.9).

The ¹H NMR signal of guanine appears as a singlet at δ 8.41 [s, 1H, H(8)]³⁵. In case of the metal complexes, the signal shifts to ca. δ 8.60. This downfield shift in organohafnium(IV)-guanine complexes is attributed to the involvement of N(7) in complexation.

In the ¹H NMR spectra of the metal complexes, the cyclopentadienyl group appeared as a multiplet in the region δ 6.9-7.0 (m, 10H). In the complexes containing phenyldithiocarbazate group as the anionic moiety, a multiplet in the region δ 7.7-8.1 (m, 5H) was observed due to five protons in the C₆H₅ moiety.

In the ¹³C NMR spectrum of pure guanine, the signals due to C(6) carbonyl and C(8) appeared at 160.3 ppm and 148.2 ppm, respectively. However, in the complexes, the former signal shifts downfield to ca. 164.0 ppm and the latter to ca. 152.0 ppm, indicating that complexation involves deprotonation of N(7) and chelation through C(6) carbonyl. The resonance signals due to C(2), C(4) and C(5) in case of the metal complexes of guanine are observed at ca. 170.8 ppm, ca. 150.2 ppm and ca. 116.3 ppm, respectively.

In the metal complexes, a resonance signal appeared at ca. 122.2 ppm due to carbons of the cyclopentadienyl rings. In case of the complexes containing anionic phenyldithiocarbazate moiety, the resonance signals observed were: ca. 153.0 ppm C(1); ca. 112.1 ppm (C(2,6)); ca. 130.1 ppm (C(3,5)) and ca. 119.2 ppm (C(4)), while the signal due to -CS₂ carbon appeared at ca. 169.0 ppm.

Fluorescence studies

The compound [(η⁵-C₅H₅)HfL₁][HgCl₃] is fluorescent in nature. Hence, fluorescence studies have been carried out for it. In accordance with the Franck-Condon principle and thermal relaxation of vibrational modes, the fluorescence spectrum is observed on the red side of the absorption spectrum in approximately mirror-image relationship. The spectrum is free from anti-Stokes effect. The pattern of the spectrum follows Levschin's rule, indicating that the geometry of the excited state is not very different from that of the ground state.

The actual radiative lifetime of the excited state, τ, is smaller than the intrinsic radiative lifetime, τᵢ, indicating the possibility of non-radiating energy
dissipation processes depopulating the excited state. Thus, fluorescence remains the dominant but certainly not the exclusive mode of emission. The non-radiative processes, i.e. inter-system crossing and internal conversion, compete with fluorescence. Therefore, Einstein’s probability of spontaneous absorption, $B_{nn}$, exceeds the corresponding probability of spontaneous emission, $A_{nm}$. The quantum yield of fluorescence, intersystem crossing and internal conversion follow the order $\phi_f > \phi_{ISC} > \phi_{IC}$. These priorities are also established by their respective rate constants.

The summation of the rate constants for all the photochemical and photophysical processes competing with fluorescence, $\Sigma K_i$, equals the sum of $K_{ISC}$ and $K_{IC}$. In the present case, there seems to be no other mode of emission, radiative or nonradiative apart from fluorescence, intersystems crossing and internal conversion. The oscillator strength, $f$, has been calculated from the relation $f = 4.31 \times 10^{-9} \int \epsilon \, d\nu$, which is valid if we assume a Lorentzian shape for the absorption band. The factor $\int \epsilon \, d\nu$ is replaced by $\epsilon_{max} \Delta \nu$, where $\Delta \nu$ is the half band width of the absorption band. The relevant data are presented in Table 2.

**Table 2 — Fluorescence spectral parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantum yield of fluorescence: $\phi_f$</td>
<td>0.60</td>
</tr>
<tr>
<td>Quantum yield for inter-system crossing: $\phi_{ISC}$</td>
<td>0.35</td>
</tr>
<tr>
<td>Quantum yield for internal conversion: $\phi_{IC}$</td>
<td>0.0086</td>
</tr>
<tr>
<td>Oscillator strength: $f$</td>
<td>0.012</td>
</tr>
<tr>
<td>Actual radiative lifetime: $\tau$ (s)</td>
<td>$5.5 \times 10^{-8}$</td>
</tr>
<tr>
<td>Intrinsic radiative lifetime: $\tau$ (s)</td>
<td>$8.9 \times 10^{-8}$</td>
</tr>
<tr>
<td>Rate const of fluorescence emission: $K_{R}$ (s^-1)</td>
<td>$1.0 \times 10^{-8}$</td>
</tr>
<tr>
<td>Rate const for inter-system crossing, $S_f \rightarrow T_f$: $K_{ISC}$ (s^-1)</td>
<td>$0.58 \times 10^{-8}$</td>
</tr>
<tr>
<td>Rate const for internal conversion: $K_{IC}$ (s^-1)</td>
<td>$0.0015 \times 10^{-8}$</td>
</tr>
<tr>
<td>Einsteins absorption probability: $B_{nm}$</td>
<td>$0.39 \times 10^{-8}$</td>
</tr>
<tr>
<td>Einsteins emission probability: $A_{nm}$</td>
<td>$0.14 \times 10^{-8}$</td>
</tr>
</tbody>
</table>

**Thermal studies**

Thermal studies have been carried out for complexes containing metal salts in the anionic species. The TG curves of guanine complexes in Fig. 1 indicate that the complexes finally decompose to give the oxides of hafnium and the metal in the anionic moiety. In the case of the complexes containing mercury in the anionic moiety, the observed weight loss corresponds to the formation of HfO$_2$ only, since HgO volatilizes at such high temperatures. Figure 2 represents the DTA curves of guanine complexes, while Fig. 3 represents their linearization curves, respectively. The mass loss data of the complexes have also been recorded. Mass loss, found (calcd): (1) 52.6 (53.8); (2) 50 (53.7); (3) 49.9 (50); (4) 74 (72.5). The order of the reaction ($n$) and the activation energy ($E_a$) of the thermal decomposition reaction have been elucidated by the
method of Coats and Redfern. The entropy of activation ($S^*$) has been calculated by the method of Zsak. The kinetic and thermodynamic parameters calculated from TG and DTA studies are given in Table 3.

The order of thermal decomposition reaction in all the complexes is found to be one. A comparison of the activation energy of complexes reveals that the $E_a$ values follow the order,

$$[(\eta^5-C_5H_5)_2HfL]^+ [CuCl_3]^- < [(\eta^5-C_5H_5)_2HfL]^+ [ZnCl_3]^- < [(\eta^5-C_5H_5)_2HfL]^+ [CdCl_3]^- < [(\eta^5-C_5H_5)_2HfL]^+ [HgCl_3]^-$$

This may be explained on the basis of the fact that larger cations are stabilized by larger anions. The complex cation $[(\eta^5-C_5H_5)_2HfL]^+$ is very large in size and the size of the M(II) ions in the anionic moiety varies in the order Cu(II) < Zn(II) < Cd(II) < Hg(II).

In case of the complex, $[(\eta^5-C_5H_5)_2HfL]^+ [HgCl_3]^-$, the large size of Hg(II) ion in the anionic moiety, helps in effective stabilization of the complex cation and therefore gives rise to a higher lattice energy. This makes thermal degradation of the complex relatively difficult and thus, the reaction involves a higher value of $E_a$. For the complex, $[(\eta^5-C_5H_5)_2HfL]^+ [CuCl_3]^-$, the small size of the Cu(II) ion in the anionic moiety leads to a relatively poor stabilization of the complex cation and to a comparatively lower lattice energy. Thus, the thermal decomposition reaction involves a lower value of $E_a$.

It is, therefore, evident that the nature of metal ion in the anionic moiety contributes to the variation in the activation energy for the thermal degradation of the complexes. The energy of activation, in turn, reflects the kinetic lability of the complexes. The compounds with lower $E_a$ values are more labile as compared to those with higher $E_a$ values.

The apparent activation entropy has a positive value for most of the complexes. Hence, thermal degradation of these complexes is a spontaneous process. Within a given series, the complex

<table>
<thead>
<tr>
<th>Complex</th>
<th>Temp. Range (K)</th>
<th>Activation Energy ($E_a$) (kJ mol$^{-1}$)</th>
<th>Activation entropy ($S^*$) (JK$^{-1}$ mol$^{-1}$)</th>
<th>$T_{max}$ (K)</th>
<th>$\Delta H$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>485-703</td>
<td>27.33</td>
<td>7.956</td>
<td>665</td>
<td>372.41</td>
</tr>
<tr>
<td>(2)</td>
<td>498-773</td>
<td>30.60</td>
<td>9.329</td>
<td>653</td>
<td>201.87</td>
</tr>
<tr>
<td>(3)</td>
<td>499-803</td>
<td>33.47</td>
<td>10.570</td>
<td>734</td>
<td>189.25</td>
</tr>
<tr>
<td>(4)</td>
<td>448-855</td>
<td>62.17</td>
<td>23.214</td>
<td>694</td>
<td>144.53</td>
</tr>
</tbody>
</table>
containing Cu(II) ion in the anionic moiety has the lowest value of \( S^* \) while the one containing Hg(II) ion has the highest. Hence, the former decomposes with lowest degree of randomness, while the latter with greatest.

The TG data of the complexes is supplemented by DTA studies. The thermal effects on DTA curves are exclusively exothermic in nature. The heat of reaction (\( \Delta H \)) for the thermal decomposition reaction has been enumerated from the DTA curves. The temperature dependent calibration coefficient has been obtained from the Currell Equation.

**Microbial assay**

Table 4 gives the growth inhibition effected by complexes containing copper(II) and mercury(II) ions in the anionic moiety of the guanine complexes against pathogenic strains of *Escherichia coli* (E.coli), *Pseudomonas aeruginosa* (P.aeruginosa) and *Zymomonas mobilis* (Z.mobilis) using the respective ligand as the standard for comparing the activities. The samples have been screened at two concentrations (25 \( \mu \)g mL\(^{-1} \) and 50 \( \mu \)g mL\(^{-1} \)) in DMF. The inhibitory power of the complexes was greater than the control. The complexes, in general, inhibited the growth of bacteria to a greater extent as the concentration increased. However, the complex containing Cu(II) ion in the anionic moiety was similar in its activity against (E.coli) as the free ligand.

The higher antibacterial activity of organohafnium(IV) ionic chelate complexes containing mercury in the anionic moiety was due to the capacity of mercurials to coordinate with sulphhydryl functions of cellular proteins and enzymes,

\[
\text{Enzyme} \overset{\text{SH}}{\rightleftharpoons} \text{Hg}^2+ \rightarrow \text{Enzyme} \overset{\text{S}}{\rightleftharpoons} \text{Hg} + 2\text{H}^+ 
\]

Active enzyme Inactive enzyme

**Table 4 — Anti-bacterial activity of guanine complexes**

<table>
<thead>
<tr>
<th>Complex</th>
<th><em>Escherichia coli</em></th>
<th><em>Pseudomonas aeruginosa</em></th>
<th><em>Zymomonas mobilis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 ( \mu )g mL(^{-1} )</td>
<td>50 ( \mu )g mL(^{-1} )</td>
<td>25 ( \mu )g mL(^{-1} )</td>
</tr>
<tr>
<td>(1)</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>(4)</td>
<td>++</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>HL</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Cp_2HfCl(_2)</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Each (+) sign indicates 2 mm section of zone of inhibition.

The antifungal activity of complexes containing mercury and the phenyldithiocarbazate group in the anionic moiety, at concentrations 25 \( \mu \)g cm\(^{-1} \) and 50 \( \mu \)g cm\(^{-1} \), against *Aspergillus niger* (A.niger) and *Aspergillus awamori* (A.awamori) is presented in Table 5. It has been observed that: (a) complexes containing the phenyldithiocarbazate group in the anionic moiety were comparatively stronger antifungal agents than those containing mercury in the anionic moiety, and (b) the former complexes showed greater inhibition at lower concentrations, while the latter were most active at higher concentrations.

The mode of fungicidal action of the dithiocarbazates involves the inhibition of hexose monophosphate oxidation pathway of carbohydrate metabolism.

**Conclusion**

The present work affords proof for the formation of N7/O6 chelate with a 6-oxopurine. This type of interaction has attracted considerable attention in recent years, since it has been proposed that such kind of complexes are biochemically active, as is the case with the complexes reported herein.

**References**
