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5-Fluorouracil and 5-fluorouracil-histidine complexes with Al^{III}, Cr^{III} and Fe^{III} ions and their antitumour activity

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Abstract—Complexes of the type [M(L-H)(OH)Cl], [Cr(L-H)$(H_2O)_2$(OH)Cl] and [M′(L-H)(L′-H)(H_2O)Cl], where L = 5-fluorouracil; L′ = histidine (HISD); M = Al^{III} or Fe^{III} and M′ = Al^{III}, Cr^{III} or Fe^{III} were synthesized and characterized. The complexes are insoluble in water and common organic solvents. 5-Fluorouracil is coordinated to the metal ion through the O atom of $C_{(4)}$=O and the N atom of $N_{(1)}$ while histidine coordinates through the O atom of $-COO^-$ and the N atom of $-NH_2$ groups. The μ_{eff} values, electronic spectral bands and ESR spectra suggest a polymeric 6-coordinate spin free octahedral stereochemistry for Cr^{III} and Fe^{III} complexes. The *in vivo* antitumour effect of 5-fluorouracil and its complexes was examined on C_3H/He mice against P815 murine mastocytoma. As evident from their T/C values Cr^{III} and Fe^{III} complexes display significant and higher antitumour activity compared to 5-fluorouracil while the Al^{III} complexes show lower activity. The *in vitro* results of the complexes on the same cells indicate that Cr^{III} and Fe^{III} complexes show higher inhibition on ^{3}H-thymidine and ^{3}H-uridine incorporation in DNA and RNA replication, respectively.
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Keywords: 5-fluorouracil; histidine complexes; aluminium(III); chromium(III); iron(III); antitumour.

Chelating agents, if present in high concentrations in cancer cells, help in diminishing the concentration of abnormal metal ions by complexing them [1]. A few substituted pyrimidines have been reported to be active as chemotherapeutic agents and seem to fit the chelate hypothesis. The most useful is 5-fluorouracil which has been increasingly employed alone or in combination with other drugs and hormones in the treatment of several tumours [2–6]. A number of bivalent transition metal complexes of 5-fluorouracil have been reported to possess significant antitumour activity *in vivo* and *in vitro*, when examined against standard tumour ascites sarcoma-180 [7].

Metal ions in biological systems may also promote the interaction of proteins and nucleic acids through the formation of ternary complexes. The formation of nucleic acid-enzyme-transition metal ternary complexes during DNA replication and RNA synthesis are known [8]. The formation of such ternary complexes may also induce selective interaction between amino acid residues and nucleic acid bases, even those far removed from the site of metal attachement. The study of ternary complexes involving 5-fluorouracil and amino acid like L-histidine may provide models for the more complicated metal-protein-nucleic acid interactions. Studies on ternary complexes of histidine with Mn^{II}, Co^{II}, Ni^{II}, Cu^{II} and Zn^{II} metal ions have been reported by a number of workers [9–12]. The ternary complexes of L-histidine with Pd^{II} showed growth-inhibitory activity against L1210 lymphoid leukemic, P388 lymphocytic leukemic, sarcoma 180 and Ehrlich ascites tumour cells [13]. Accordingly the synthesis, characterization and antitumour activity of Al^{III}, Cr^{III} and Fe^{III} complexes with the above ligands are discussed in this paper.

EXPERIMENTAL

Materials

All the chemicals used were of AnalaR or equivalent grade. The metal chlorides employed were of E. Merck grade. 5-Fluorouracil was purchased from Aldrich and L-histidine from Sisco Research Laboratory

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(SRL), India. ^{3}H-Thymidine and ^{3}H-uridine used for antitumour activity were obtained from the Bhabha Atomic Research Centre, Bombay. The mice used for antitumour activity were obtained from the Tata Institute of Fundamental Research, Bombay.

Preparation of the complexes

5-Fluorouracil complexes. 1 mmol of 5-fluorouracil (0.1300 g) in each case was suspended in 35 cm^3 ethanol and 15 cm^3 triethyl orthoformate and refluxed at about 80°C for about 1/2 h to dissolve. To this solution 1 mmol each of $AlCl_3 \cdot 6H_2O$ (0.2415 g), $FeCl_3 \cdot 6H_2O$ (0.2705 g) or $CrCl_3 \cdot 6H_2O$ (0.2665 g) in 10 cm^3 hot ethanol was added and refluxed for 2–3 h at the boiling point. A clear solution was thus obtained. From this solution, the metal complexes were precipitated by raising the pH between 5–6 by adding 0.2 N NaOH dropwise. The precipitates were digested, filtered, washed with ethanol and ether successively and dried at 50°C.

5-Fluorouracil-histidine complexes. Using the same quantities of 5-fluorouracil and metal salts and conditions of precipitation maintained as above, 1 mmol (0.1550 g) L-histidine, dissolved in 10 cm^3 of hot distilled water was added in each case of precipitation. On reacting with L-histidine the precipitate was again dissolved. The resultant solution was concentrated over a water bath to 50% of its volume. The mixed ligand complexes were precipitated by adding ether. The precipitates were filtered and washed with ethanol and finally with ether and dried at 50°C.

Analysis and instrumentation

The metal contents of the complexes were estimated after dissolving the complexes in very dilute HNO_3 and titrating with excess 0.01 M EDTA at pH 5 by following the standard procedures [14,15]. Analysis of C, H and N were carried out microanalytically on a Perkin-Elmer 240C model microanalyzer.

Room temperature magnetic susceptibilities of the complexes were determined with a Faraday type balance (Cahn magnetic susceptibility apparatus) using $[CoHg(SCN)_4]$ as calibrant and correcting the experimental values for diamagnetism [16]. Electronic spectra of the complexes were recorded on a Shimadzu 160A UV-visible recording spectrophotometer. Infrared spectra of the ligands and their complexes were recorded in KBr pellets on a Perkin-Elmer 783 infrared spectrophotometer. X-band ESR spectra of chromium(III)-5-fluorouracil-histidine complex was recorded on a JES-ME-3X type spectrometer in powder state at room temperature and liquid nitrogen temperature using DPPH as g marker (g = 2.00238).

Antitumour activity evaluation

In vivo *testing.* C_3H/He mice of either sex of about 6–8 weeks old and average body weight of 20 g were used for *in vivo* antitumour activity against P815 (murine mastocytoma) test system. Six animals were used for each set of experiments. An intraperitoneal injection of 2×10^5 tumour cells was given to each mouse at weekly intervals. Fine suspensions of the test compounds, prepared in 0.89% saline (NaCl), were injected once a week after two days of tumour transplantation at the doses of 12.5 and 25 mg/kg body weight of mice. The same volume of sterile saline was injected in the control set.

Therapeutic effectiveness of each compound against tumour-bearing mice was assessed from T/C percentage which was calculated as

$$\% \text{ T/C} = \frac{\text{Mean life span of treated mice*}}{\text{Mean life span of untreated mice}} \times 100$$

In vitro *testing.* The P815 murine mastocytoma tumour cell suspension (1×10^6 cells/cm^3) was prepared in complete medium containing tissue culture of RMPI 1640 supplemented with antibiotics, penicillin, streptomycin and 10% heat inactivated fetal calf serum. For determination of effects of various compounds on DNA replication, duplicate culture plates (NUNC, Denmark) containing 96 wells in each plate were taken and 2×10^5 tumour cells were added in each cell well. The test compounds at different doses (1, 5 and 10 μg/cm^3) were added in one set of culture plates while the other set was without test compound. After 24 h in a CO_2 incubator at 37°C, the cells were washed thrice with RMPI 1640 culture medium by centrifugation for 10 min. The cell pellets were resuspended in 0.2 cm^3 complete medium (RMPI 1640 + antibiotics + 10% heat inactivated fetal calf serum) containing 0.5 μCi/cm^3 ^{3}H-thymidine for 18 h reincubation. Again cell pellets were centrifuged and supernatant liquid was discarded, washed thrice with balance salt solution (phosphate buffered normal saline). The cell pellets were digested with 0.5% sodium dodecyl sulfate (SDS) and the lysate was counted for radioactivity in LKB β-liquid scintillation counter. The percentage inhibition of incorporation of ^{3}H-thymidine in DNA was calculated as

$$\% \text{ inhibition} = 1 - \frac{\text{CPM in treated tumour cells}}{\text{CPM in untreated tumour cells}} \times 100$$

where CPM = counts per minute of radioactivity of ^{3}H-thymidine.

For the effects of compounds on RNA synthesis ^{3}H-uridine (0.5 μCi/cm^3) was used in place of ^{3}H-thymidine and other procedures are same as discussed above.

RESULTS AND DISCUSSION

It appears from the analytical data of the complexes (Table 1) that all the 5-fluorouracil complexes display

*Excluding tumour free survivor.

1 : 1 (M : L) stoichiometry and the mixed ligand complexes exhibit 1 : 1 : 1 (M : L : L′) stoichiometry. The course of reactions may be as follows:

$$MCl_3 \cdot 6H_2O + L \longrightarrow M(L\text{-}H)Cl_2 \cdot xH_2O + HCl$$

$$M(L\text{-}H)Cl_2 \cdot xH_2O \xrightarrow[pH\ 5\text{–}6]{NaOH} [M(L\text{-}H)(OH)Cl] + NaCl$$

$$\text{or } [M(L\text{-}H)(H_2O)_2(OH)Cl]$$

$$[M(L\text{-}H)(OH)Cl] + L' \longrightarrow [M(L\text{-}H)(L'\text{-}H)(H_2O)Cl]$$

The Al^{III} complexes are colourless while the Fe^{III} and Cr^{III} complexes are coloured. They are insoluble in water and other common organic solvents like ethanol, benzene, chloroform, carbon tetrachloride, acetone, acetonitrile, pyridine, ether, DMF and DMSO. The Al^{III} complexes melt between 276–280°C while the Fe^{III} and Cr^{III} complexes decomposed without melting above 300°C. A significant weight loss occurs between 140–160°C in most of the complexes (except Al^{III} and Fe^{III}-5-fluorouracil complexes), indicating that they contain coordinated water molecules.

The insolubility and non-melting nature of the complexes suggest that they are polymeric compounds [17]. Therefore, solution studies like molar conductance, NMR and single crystal X-ray diffraction studies were not possible.

Magnetic moment and electronic spectra

Both the Cr^{III} complexes show μ_{eff} values 3.96 and 3.78 B.M. respectively, corresponding to three unpaired electrons suggesting octahedral stereochemistry [18]. The Fe^{III}-5-fluorouracil and Fe^{III}-5-fluorouracil-histidine exhibit μ_{eff} values 5.57 and 5.54 B.M. respectively. Both these values are slightly less than the corresponding value for 5 unpaired electrons [18] (μ_{eff} = 5.94 B.M.) indicating possible magnetic exchange interaction due to polymerization or OH bridging.

Octahedral Cr^{III} complexes are expected to show three spin allowed *d-d* transitions *viz.* $^4A_{2g}(F) \longrightarrow {}^4T_{2g}$ (ν_1), $\longrightarrow {}^4T_{1g}$ (ν_2) and $\longrightarrow {}^4T_{1g}(P)$ (ν_3) [19]. In both the Cr^{III} complexes in this study only two bands are observed at 532, 526 nm (ν_1) and 343, 355 nm (ν_2) respectively. The ν_3 band, expected below 300 nm overlaps with the ligand bands or L⟶M charge transfer bands and is, therefore, not assigned [19]. A slight splitting in the above bands of Cr^{III} complexes is also observed which may partly be due to low symmetry hexacoordinated configurations and partly due to the presence of different chromophores in the polymeric complexes [20].

The first two bands appearing in Fe^{III}-5-fluorouracil and Fe^{III}-5-fluorouracil-histidine complexes at 495, 490 nm and 405, 392 nm respectively may be assigned to $^6A_{1g} \longrightarrow {}^4T_{1g}$ and $\longrightarrow {}^4T_{2g}$ transitions. The third band at 342 and 348 nm for the two complexes respectively may be due to the L⟶M charge transfer which obscures the low intensity *d-d* absorption bands.

ESR spectra

The ESR spectra of the powdered sample of Cr^{III}-5-fluorouracil-histidine complex at room temperature (300 K) and liquid nitrogen temperature (77 K) show a strong broad band with no evidence of fine structure in each case. These results merely indicate strong dipolar coupling between the paramagnetic ions, as expected for a polymeric structure. However, g_{iso} = 1.949 at R.T. and 1.962 at LNT suggest an octahedral environment around Cr^{III} [21].

IR spectra

5-Fluorouracil complexes. Some important IR frequencies of 5-fluorouracil and its complexes are given in Table 2. There are several absorption bands in the region 3500–3000 cm^{-1} which may be attributed to —NH and —OH stretching modes. The νO—H band appearing at 3390, 3400 and 3400 cm^{-1} in Al^{III}, Fe^{III} and Cr^{III} complexes, respectively, indicate the presence of a water molecule or O—H group [22]. The presence of νM—O (aqua) bands in the lower region of Cr^{III}-5-fluorouracil complex confirm the bonding of water molecules in the complex [22]. The presence of a medium broad band in the region *ca* 950 cm^{-1} in Al^{III} and Fe^{III} complexes may be assigned as OH bridging, suggesting an OH bridged polymeric structure for the complexes [22].

The $\nu C_{(4)}{=}O$ band occurring at 1695 cm^{-1} in 5-fluorouracil is shifted considerably towards a lower frequency (*ca* 35–50 cm^{-1}) in all the complexes, suggesting the coordination of the $C_{(4)}{=}O$ group with the metal [20]. The $\nu C_{(2)}{=}O$, νC—F and $\delta N_{(3)}$—H bands appear at 1710, 1470 and 1430 in free 5-fluorouracil. These bands either do not shift or show slight shifts in the metal complexes indicating that these groups are not taking part in coordination. The $\delta N_{(1)}$—H band of 5-fluorouracil at 1512 cm^{-1} disappears in all the metal complexes suggesting the deprotonation of $N_{(1)}H$ proton and bonding of $N_{(1)}$ nitrogen with the metal. The metal–oxygen stretching vibrations appear in the region 225–245 cm^{-1} for six and at 276 cm^{-1} for four coordination number [23]. The presence of characteristic bands in the region 240–248 cm^{-1} strongly favour coordination number six for all complexes. The νM—N and νM—Cl bands are tentatively assigned in the lower region. In the Al^{III} and Fe^{III} complexes, νM—Cl occur at significantly lower wave numbers relative to the Cr^{III} complex. This may be due to the presence of bridging chloro ligand in these complexes [24].

5-Fluorouracil-histidine complexes. In all the 5-fluorouracil-histidine mixed ligand complexes 5-fluorouracil shows similar trends of bonding and shifts in the affected group frequencies as described earlier for

Table 1. Analytical and electronic spectral data

Complex	Colour	Decomp. temp. (°C)	Found (Calc.) (%) M	Cl	C	H	N	μ_{eff} (B.M.)	λ_{max} (nm)
[Al(L-H)(OH)Cl]	White	276	12.8 (12.9)	17.0 (17.0)	22.8 (23.0)	1.5 (1.4)	13.3 (13.4)	—	—
$[Cr(L\text{-}H)(H_2O)_2(OH)Cl]$	Deep green	>300	19.1 (19.3)	13.0 (13.2)	17.7 (17.8)	2.6 (2.6)	10.3 (10.4)	3.96	532,343
[Fe(L-H)(OH)Cl]	Light brown	>300	23.4 (23.6)	14.8 (14.9)	20.0 (20.2)	1.2 (1.2)	11.7 (11.8	5.57	495,405, 342
$[Al(L\text{-}H)(L'\text{-}H)(H_2O)Cl]$	Dirty white	280	7.3 (7.4)	9.6 (9.7)	32.8 (33.0)	3.3 (3.3)	19.1 (19.2)	—	—
$[Cr(L\text{-}H)(L'\text{-}H)(H_2O)Cl]$	Blue green	>300	13.2 (13.4)	9.0 (9.1)	30.7 (30.9)	3.1 (3.1)	17.9 (18.0)	3.78	526,355
$[Fe(L\text{-}H)(L'\text{-}H)(H_2O)Cl]$	Dark brown	>300	14.1 (14.2)	9.0 (9.0)	30.4 (30.6)	3.1 (3.0)	17.8 (17.8)	5.54	490,392, 348

L = 5-fluorouracil; L′ = histidine.

Table 2. Important IR spectral data of 5-fluorouracil complexes

Compound	νOH	$\nu C_{(2)}=O$	$\nu C_{(4)}=O$	$\delta N_{(1)}—H$	$\delta N_{(3)}—H$	νM—O(aqua)	νM—N	νM—O	νM—Cl
5-Fluorouracil	—	1710s	1695s	1512m	1430m	—	—	—	—
[Al(L-H)(OH)Cl]	3390b	1705s	1650s	—	1435m	—	280w	240w	320w
$[Cr(L\text{-}H)(H_2O)_2(OH)Cl]$	3400b	1708s	1660s	—	1432s	430m	276w	242w	370w
[Fe(L-H)(OH)Cl]	3400b	1710s	1645s	—	1435m	—	265w	248w	315w

Table 3. Important IR spectral data of 5-flourouracil-histidine complexes

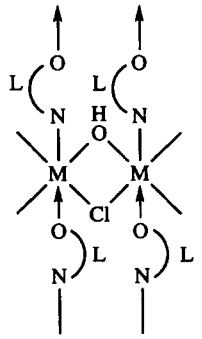
Compound	νOH	νNH_3^+	$\nu C_{(2)}=O$	$\nu C_{(4)}=O$	νCOO^-	νC—N	νC=N	$\delta N_{(3)}—H$	νM—O(aqua)	νM—Cl
Histidine	—	3071m	—	—	1573s	1520m	1470s	—	—	—
$[Al(L\text{-}H)(L'\text{-}H)(H_2O)Cl]$	3420b	—	1708s	1655s	1552w	1515m	1470s	1432m	435w	345w
$[Cr(L\text{-}H)(L'\text{-}H)(H_2O)Cl]$	3410b	—	1712s	1650s	1548m	1518w	1470s	1430m	410w	360w
$[Fe(L\text{-}H)(L'\text{-}H)(H_2O)Cl]$	3398b	—	1710s	1662s	1547m	1520s	1470s	1428w	449w	349w

5-flourouracil complexes. In addition, some new vibrations due to histidine coordination are observed in these complexes. The bands due to C—N and C=N groups of the imidazole residue of histidine do not shift in the spectra of the metal complexes (Table 3) suggesting non-involvement of imidazole residue in coordination. The νCOO^- bands of histidine show a shift towards lower wave number in complexes providing evidence that histidine is bonded to the metal ion through the carboxylic group [25]. Histidine also shows a $\nu N—H$ of $—NH_3^+$ at 3071 cm^{-1}, which disappears on coordination suggesting coordination of histidine through the nitrogen of the amino group [26]. This is further supported by the disappearance of $\delta N—H$ of $—NH_3^+$ at 1503 cm^{-1} in histidine on complex formation.

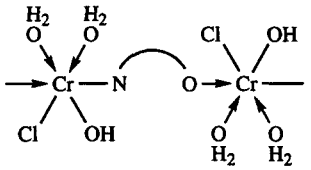
Based on the above discussions, general structures for the metal complexes in Fig. 1 are proposed.

Antitumour properties

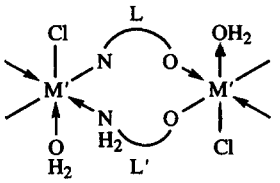
In vivo *effect*. The *in vivo* effect of the ligand, 5-fluorouracil and their Al^{III}, Fe^{III} and Cr^{III} complexes in this study on P815 murine mastocytoma were evaluated on the basis of their percent T/C value. A T/C value of 115 indicates a significant activity whereas, 125 indicates that the compound is very useful for testing on other tumour systems [27]. At the dose of 25 mg/kg body weight, the compounds were cytotoxic and therefore, the results obtained at the dose of 12.5 mg/kg body weight were recorded. The experimental data (Table 4) indicate that both the Fe^{III} complexes show a significant antitumour activity having equal percent T/C (168) values which are highest among all other complexes in the present study at the above dose. The Cr^{III}-5-fluorouracil and Cr^{III}-5-fluorouracil-histidine complexes show a slightly higher activity



[M(L-H)(OH)Cl]

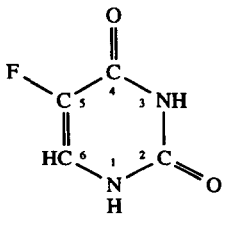


[Cr(L-H)(H_2O)$_2$(OH)Cl]

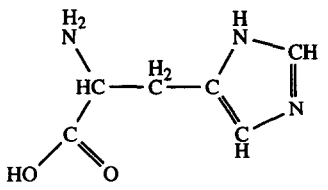


[M'(L-H)(L'-H)(H_2O)Cl]

Where M = Al (III) or Fe (III); M' = Al (III), Cr (III) or Fe (III)



L = 5–Fluorouracil



L' = Histidine

Fig. 1.

Table 4. Antitumour activity *in vivo*

Compound	Dosage mg/kg body weight	Mean life span of nonsurvivors T/C	% T/C
5-Fluorouracil	12.50	34/22	154
[Al(L-H)(OH)Cl]	12.50	14/22	64
[Cr(L-H)$(H_2O)_2$(OH)Cl]	12.50	35/22	159
[Fe(L-H)(OH)Cl]	12.50	37/22	168
[Al(L-H)(L′-H)(H_2O)Cl]	12.50	14/22	64
[Cr(L-H)(L′-H)(H_2O)Cl]	12.50	35/22	159
[Fe(L-H)(L′-H)(H_2O)Cl]	12.50	37/22	168

Table 5. Antitumour activity *in vitro*

Compound	% inhibition of 5 $\mu g/cm^3$	^{3}H-thymidine 1 $\mu g/cm^3$	% inhibition of 5 $\mu g/cm^3$	^{3}H-uridine 1 $\mu g/cm^3$
5-Fluorouracil	48.15	42.96	34.81	31.13
[Al(L-H)(OH)Cl]	48.20	43.00	36.17	32.83
[Cr(L-H)$(H_2O)_2$(OH)Cl]	87.28	84.66	71.74	66.08
[Fe(L-H)(OH)Cl]	86.36	81.62	62.74	61.20
[Al(L-H)(L′-H)(H_2O)Cl]	48.20	43.25	35.13	31.74
[Cr(L-H)(L′-H)(H_2O)Cl]	86.26	80.15	64.82	49.26
[Fe(L-H)(L′-H)(H_2O)Cl]	78.42	74.23	56.12	38.12

than 5-fluorouracil with equal percent T/C (159) value, however, both the Al^{III} complexes show poor activity.

5-Fluorouracil itself shows a significant antitumour activity (% T/C = 154) but inspite of its lower concentration by weight, in the complexes, the activity is increased on complexation with Fe^{III} and Cr^{III}. This may be due to the metal effect because similar complexes with Al^{III} (% T/C = 64) are inactive in the present study.

In vitro *effect*. The data obtained for the inhibitory effect on DNA and RNA replication *in vitro* (Table 5) of the compounds indicate that both the Cr^{III} and Fe^{III} complexes show significant activity at the dose of 1 and 5 $\mu g/cm^3$, but Cr^{III} complexes show slightly higher *in vitro* antitumour activity than Fe^{III}. All the complexes show a dose dependent activity. At the dose of 10 $\mu g/cm^3$ the complexes were cytotoxic. All the complexes show maximum inhibition of DNA and RNA replication at the dose of 5 $\mu g/cm^3$. The Al^{III} complexes were again found to have a lesser inhibitory effect on DNA as well as RNA replication than the Cr^{III} and Fe^{III} complexes.

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