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Design of Antineoplastic Ruthenium Complexes and their Pharmacological Evaluation

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Based on the results obtained from geometry optimization, using molecular mechanics and ZINDO/1 force fields, of some known antitumor ruthenium compounds we designed five ruthenium chelates and synthesized them. Effects of these compounds on the growth of a transplantable murine tumor and the life span of the hosts are studied. Remarkable decrease in tumor volume and viable ascitic cell count is observed ($p<0.05$). Treatment with the ruthenium compounds prolonged the lifespan of Ehrlich Ascites Carcinoma (EAC) tumor bearing mice. Tumor inhibition by the ruthenium chelates was followed by improvements in hemoglobin, RBC and WBC values. Thus the results suggest that these ruthenium chelates have significant antitumor property against experimental murine tumors and it does not adversely affect the hematological profile of the host.

Clinical studies of platinum complexes have incited the development of a number of non-platinum compounds with metal ions such as germanium (IV), titanium(IV), tin(IV), gallium(III) or ruthenium(III) which exhibit antitumour activity in *in vitro* and *in vivo* models. Among these the ruthenium complexes have been studied most extensively. Some of these complexes have entered or are about to enter, clinical trials. Although platinum and non-platinum complexes are potent antineoplastic agents, they do have serious side effects (e.g. myelotoxicity, nephrotoxicity and neuropathy) that are due to their reactions with cellular components of healthy tissues. The substitution of the central metal atom presents an opportunity for obtaining complexes that are effective against tumors with low side effects. Some classic ruthenium compounds such as $Ru(DMSO)_4Cl_2$[1], $Ru(NH_3)_4Cl_2$[2], $ImH[Ru(Im)_2Cl_4]$[3] and $ImH[Ru(Im)(DMSO)Cl_4]$ (NAMI-A)[4] are well known antitumor agents. Besides these, the tris-chelates of ruthenium with bidentate ligands show intercalative properties with the DNA[5]. Our present aim was to synthesize a wide range of complexes of ruthenium and explore the structural features as well as the contribution of the ligands that are responsible for antitumor activity. To study the molecular and structural features of the designed molecule some scientists used partition of energy methods (PEM). According to PEM total energy is viewed as the combined sum of energies such as bond deformation, angle deformation and hydrophobic interaction. Computation of all these energy contributions and their subsequent minimization lead to minimum energy confirmation of a molecule. To explore the possibility of finding suitable antineoplastic agents through molecular mechanics and ZINDO/1 simulations using HYPERCHEM®, an attempt has been made to develop few ruthenium complexes on the basis of energy minimization. This communication deals with the synthesis and antineoplastic activity on EAC-treated mice and also the toxic activity of five ruthenium co-ordinated complexes. After energy minimization, we have selected these compounds for experiment.

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MATERIALS AND METHODS

A series of new Ruthenium complexes were synthesized. These compounds are $[Ru(His)_2Cl_4](HisH)$,

$[Ru(Nic)_2Cl_4](Nich)$, Ru-aniline complex, Ru-2,5-dimethoxyaniline complex, Ru-1,2-phenylenediamine complex, Ru-2,5-dichloroaniline complex, $[Ru(Bpy)_2(Im)_2]Cl_2$ and $[Ru(Bpy)_2(Nic)_2Cl_2$. Among these $[Ru(Nic)_2Cl_4](NicH)$, Ru-aniline complex, Ru-2,5-dimethoxy aniline complex, Ru-1,2-phenylenediamine complex, Ru-2,5-dichloroaniline complex (where His-Histidine, Nic-Nicotinic acid, Bpy-Bipyridine and Im-Imidazole) were synthesised and biological evaluation was performed.

Synthesis:

Commercially available $RuCl_3xH_2O$ was purified to obtain a homogeneous Ru(III) solution by the method of Keppler *et al.*[3] $RuCl_3$.(1g) was suspended in a mixture of 120 ml of ethanol and 120 ml of (1N) HCl and refluxed for 3 h. Then the volume was reduced to 90 ml and (1N) HCl was further added to give a final volume of 120 ml. To a 10 ml portion of this solution was rapidly added 2.0 g of nicotinic acid in 1 ml of 6 N HCl. And the mixture was stirred for a few minutes, then it was kept below 0°. Crystals of the unreacted ligand were filtered off. The solution was vacuum evaporated and purified by precipitation from acidified ethanol. Yield 80%. Formation of the compound was checked by IR and elemental analysis of nitrogen (Table 1). The compounds $[Ru(Bpy)_2(1m)_2]Cl_2$ and $[Ru(Bpy)_2(Nic)_2]Cl_2$ were prepared from $Ru(DMSO)_4Cl_2$[6]. The yellow crystalline solid $Ru(DMSO)_4Cl_2$ was refluxed successively in chloroform and DMF with 2 equivalents of bipyridine. The dark brown crystalline solid formed was then refluxed in dry methanol under nitrogen atmosphere with an equivalent quantity of ligand in each case to give the two compounds.

Treatment protocol:

Albino Swiss mice (18-20 g body weight) were maintained in identical laboratory conditions and given standard food pellets (Hindustan Lever) and water *ad libitum*. LD_{50}[7] values of Ru-1 (Bis nicotinic acid tetrachloro ruthenate), Ru-II (Bis aniline tetra chloro ruthenate), Ru-III (Bis 2,5-dimethoxy aniline tetrachloro ruthenate), Ru-IV (Bis-1,2-phenylenediamine tetrachloro ruthenate) and Ru-V (Bis-2,5-dichloro aniline tetrachloro ruthenate) are 350 mg/kg, 79.43 mg/kg, 63.09 mg/kg, 150 mg/kg and 132.22 mg/kg body weight respectively. The animals were divided into 8 groups each containing 10 mice. Animals of groups 1-3 were kept as saline control (5 ml/kg body weight i.p.), Ehrlich Ascites Carcinoma (EAC) control ($2x10^6$ EAC cells/mouse i.p.) and EAC ($2x10^6$ EAC cells/mouse i.p.) + propylene glycol respectively. Ru-1 was dissolved in water and Ru-II, Ru-III, Ru-IV and Ru-V were dissolved in propylene glycol and administered (i.p.) at a dose of 2 mg/kg body weight in group 4, 5, 6, 7 and 8 respectively. All compounds were administered daily for 9 days starting 24 h after tumor transplantation. Five animals from each group were sacrificed 24 h after the last dose and the ascitic fluid volume, ascitic cell count and hematological parameters were noted. Mean survival time (MST) for remaining 5 mice of each group was noted for 6 w.

Ascites volume was noted by taking it in a graduated centrifuge tube and packed cell volume determined by centrifuging at 1000 g for 5 min. Viability of ascitic cells were checked by trypan blue (0.4% in normal saline) dye exclusion test and the count was taken in Neubauer counting chamber. The effect of the Ruthenium compounds on

TABLE 1: PHYSICAL PROPERTIES OF RU COMPLEXES

Compounds	IR absorption (cm^{-1})	Elemental analysis of Nitrogen		Solubility
		Theoretical(%)	Experimental (%)	
RUI	1410 (C-N)	7.31	7.30	Water
RUII	3050 (C-H) 1150 (C-N) 1590 (N-H)	7.23	6.52	Propylene glycol
RUIII	1500 1210 (C-N) 1030	6.32	6.30	Propylene glycol
RUIV	1580 (N-H)	10.5	11.12	Propylene glycol
RUV	1280 (C-N) 3320 (N-H)	6.52	7.23	Propylene glycol

tumor growth was monitored by recording the mortality daily for 6 w and percentage increase in life span (%ILS) was calculated.

ILS(%) = [(Mean survival of treated group) (Mean survival of control group)-1] x 100

Hematological studies[8]:

Blood was obtained from tail vein, 24 h after the last dose. For total count, blood was drawn into RBC or WBC pipettes in proper dilution and counted in Neubauer counting chamber. Hemoglobin concentration was determined by Sahli's Hemoglobinometer method. Differential count of leukocytes was done on freshly drawn blood film using Leishman's stain.

Chronic toxicity study:

For chronic toxicity studies, the mice were divided into five groups containing 10 mice in each group. Compounds were injected (i.p.) once a week for 4 w at a dose level of 2 mg/kg body weight. Group I received normal saline (0.9% NaCl, w/v; 5 ml/kg) once in a week for 4 w. Animals from each group were decapitated after 24 h of the last dose. Serum was seperated from clotted blood for the estimation of SGOT[9], SGPT[9] and Alkaline phosphate. Heparinised whole blood was taken for estimation of urea[10], creatinine[10], total protein[10] and cholesterol[10]. The data was statistically analyzed by Student's 't' Test[11].

RESULTS AND DISCUSSION

Results are summarized in Tables 1, 2 and in fig. 1a. 1b, 2a, 2b and were analysed statistically by Student's unpaired 't' test and statistical significance were considered only when $p<0.05$.

Ru-nicotinic acid complex, Ru-aniline complex and Ru-2,5-dimethoxy aniline complex significantly reduced ascitic fluid volume. Ru-aniline complex and Ru-2,5-dimethoxy aniline complex reduced the percentage of viable ascitic cells to 47 and 48% respectively in the treated groups as compared to 93% in the EAC control. Ru-nicotinic acid and Ru-2,5-dichloroaniline complexes increase the life span of the EAC treated mice by 112% and 100% respectively. The Ru complexes increased RBC count and hemoglobin content and decreased WBC count to near normal values, in EAC bearing mice. Ru-complexes marginally altered SGOT, SGPT values. Ru-2,5-dichloroaniline significantly increased alkaline phosphatase. Increased urea and creatinine content in blood have been observed in Ru-1,2-phenylenediamine complex.

The results of the present study clearly demonstrates the tumor inhibitory activity of the Ru chelates against

TABLE 2: ANTINEOPLASTIC ACTIVITY OF THE RU COMPLEXES AGAINST EAC BEARING MICE

Parameter	Normal saline (5 ml/kg)	EAC only ($2x10^6$ /mouse)	Vehicle control (5 ml/kg)	RUI (2 mg/kg)	RUII (2 mg/kg)	RUIII (2 mg/kg)	RUIV (2 mg/kg)	RUV (2 mg/kg)
Total body weight (g)	18.0	22.0	21.9	19.2	18	21.0	22	22.2
Mean survival time (days)	—	15.5	15	33	30	29	27	31
% ILS	—	—	—	112	93	87	74	100
Tumor volume (ml)	—	5.26±0.20	4.76±0.04	2.7±0.02	2.5±0.8	2.8±0.02	3.75±0.3	3.0±0.9
% Viable cells in ascites	—	93.6	92.8	51.1	47.3	48.6	51.5	67.5

Significant at $p<0.05$, All 'p' values are calculated with vehicle control

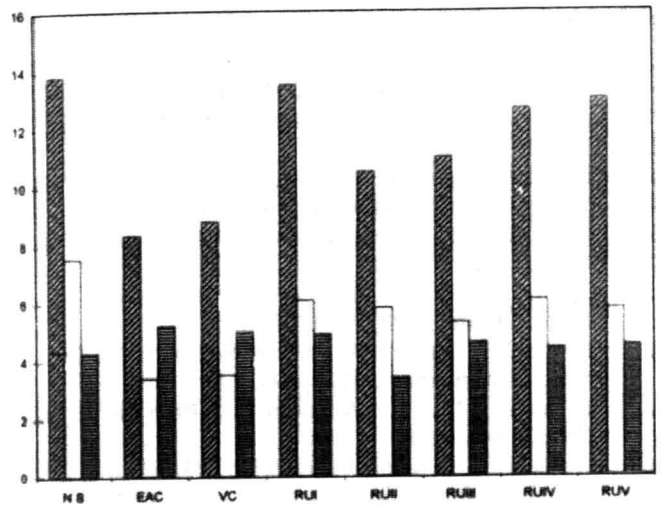

Fig. 1a. Effect of Ruthenium chelates (2 mg/kg body weight) on hemoglobin content (mg/dl) ▨ , RBC count (x10⁶) □ and WBC count (x 10³) ▤ in EAC bearing mice

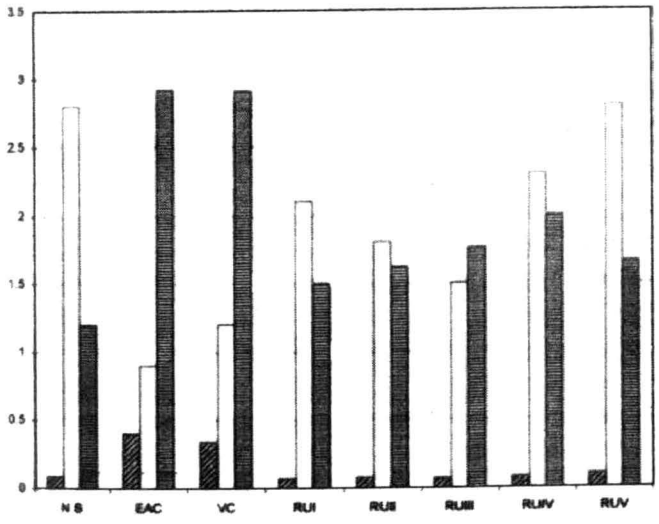

Fig. 1b: Effect of Ruthenium chelates (2 mg/kg body weight) on eosinophil (x10³) ▨, neutrophil (x10³) □ and lymphocyte (x10³) ▤ in blood of EAC bearing mice.

transplantable murine tumor cell line. The mechanism by which these compounds mediate its antitumor effect is still to be elucidated. In the EAC bearing mice cells are present in the peritoneal cavity and the compounds were administered directly into the peritoneum. Thus tumor inhibition might be due to direct effect of the compounds on tumor cells. The effect of these compounds on DNA synthesis is yet unknown but certain structurally-related

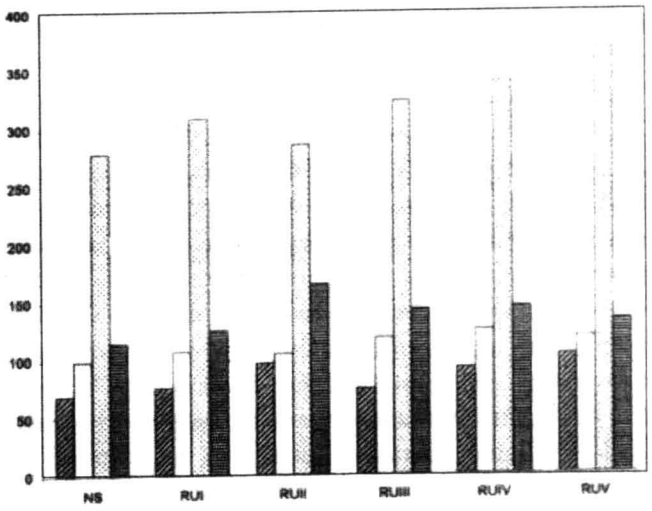

Fig. 2a: Effect of Ruthenium chelates (2mg/kg body weight) on the levels of serum enzymes SGOT (units/ml of serum) ▨ , SGPT (units/ml of serum) □, Alkaline phosphatase (units/ml of serum) ⁞⁞ and cholesterol (mg/100 ml of blood) ▤ in mice after 6 w of treatment

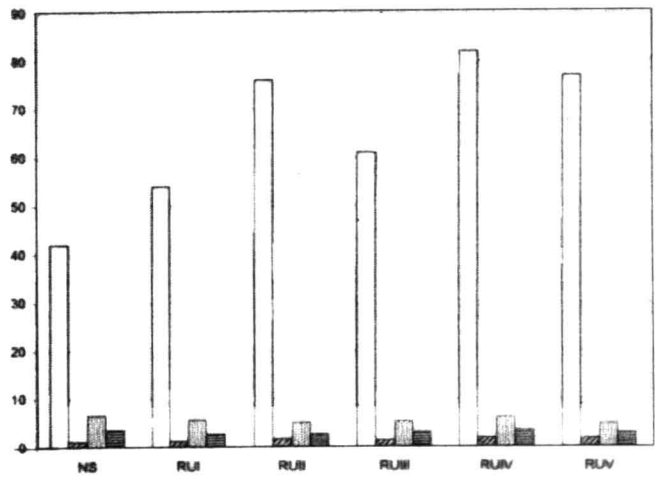

Fig. 2b: Effect of Ruthenium chelates (2 mg/kg body weight) on the blood levels of urea (mg/100 ml of blood) □ , creatinine (mg/100 ml of blood) ▨ , protein (mg/100 ml of blood) ⁞⁞ and albumin (mg/100 ml of blood) ▤ in mice after 6 w of treatment.

tris chelates of ruthenium are reported to have DNA binding property *in vitro*[5]. Likewise the action of the synthesized compounds could also be mediated *via* its effect, if any, on the DNA. Myelosupression is a frequent and

major complication of cancer chemotherapy[11]. Compared to the pretreatment values in EAC, Ru compound treatment and subsequent tumor inhibition resulted in appreciable improvements in hemoglobin content, RBC and WBC counts. These observations assume great significance as anemia is a common complication in cancer[11] and the situation aggravates further during chemotherapy since a majority of antineoplastic agents exert suppressive effects on erythropoiesis[12] and thereby limiting the use of these drugs. The improvements in hematologic profile of the tumor bearing mice following the treatment with ruthenium compounds could be secondary to tumor regression or due to the action of the compounds itself. In any case, the results of the present study are encouraging as these compounds exhibit significant reduction in the tumor burden and caused prolongation of lifespan of the hosts. Improvements, rather than aggravation, of tumor associated hematological complications such as anemia and bone marrow suppression was also noticed.

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