Eleven ruthenium complexes of the type Ru(L)$_2$Cl$_2$ (where L=2,2’-bipyridine (bpy)/ 1,10-phenanthroline (phenyl)/ dimethylsulfoxide (DMSO)) with ligands L$_j$= HBT, FC1-HBT, INH, NO$_2$-MPC, OCH$_3$-MPC, N(CH$_3$)$_2$-MPC, CI-MPC (where HBT=2-hydrazinyl-1,3-benzoiazole, FC1-HBT =5-chloro-6-fluoro-2-hydrazinyl-1,3-benzoiazole, INH=N-2-oxo-1,2-dihydro-3H-indol-3-ylidenepyridine-4-carbo-hydrazide, NO$_2$-MPC=N(4-nitrophenyl)-methylidene-pyridine-4-carboxylhydrazide, OCH$_3$-MPC=N(4-methoxyphenyl)methylidene-pyridine-4-carboxylhydrazide, N(CH$_3$)$_2$-MPC=N(4-dimethylaminophenyl)methylidene-pyridine-4-carboxylhydrazide, CI-MPC=N(4-chlorophenyl)methylidene-pyridine-4-carboxylhydrazide. The title complexes were subjected to in vitro cytostatic activity testing against the human cervix carcinoma HeLa and T-lymphocyte CEM cell lines, and the murine leukemia tumor cell line L1210. The most active ruthenium complex TKA-9 [Ru(phen)$_2$(N(CH$_3$)$_2$-MPC)] revealed a cytostatic activity of 16 μM against CEM, 20 μM against L1210 and 5.5 μM against HeLa tumor cells. All complexes were also tested for antiviral activity against a wide variety of DNA and RNA viruses, but found not to display selective activity at subtoxic concentrations.

Key words: Ruthenium complex, Cytotoxicity, Antiviral, Ligands, MLCT

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Eleven ruthenium complexes of the type Ru(L)$_2$Cl$_2$ (where L=2,2’-bipyridine (bpy)/ 1,10-phenanthroline (phenyl)/ dimethylsulfoxide (DMSO)) with ligands L$_j$= HBT, FC1-HBT, INH, NO$_2$-MPC, OCH$_3$-MPC, N(CH$_3$)$_2$-MPC, CI-MPC (where HBT=2-hydrazinyl-1,3-benzoiazole, FC1-HBT =5-chloro-6-fluoro-2-hydrazinyl-1,3-benzoiazole, INH=N-2-oxo-1,2-dihydro-3H-indol-3-ylidenepyridine-4-carbo-hydrazide, NO$_2$-MPC=N(4-nitrophenyl)-methylidene-pyridine-4-carboxylhydrazide, OCH$_3$-MPC=N(4-methoxyphenyl)methylidene-pyridine-4-carboxylhydrazide, N(CH$_3$)$_2$-MPC=N(4-dimethylaminophenyl)methylidene-pyridine-4-carboxylhydrazide, CI-MPC=N(4-chlorophenyl)methylidene-pyridine-4-carboxylhydrazide. The title complexes were subjected to in vitro cytostatic activity testing against the human cervix carcinoma HeLa and T-lymphocyte CEM cell lines, and the murine leukemia tumor cell line L1210. The most active ruthenium complex TKA-9 [Ru(phen)$_2$(N(CH$_3$)$_2$-MPC)] revealed a cytostatic activity of 16 μM against CEM, 20 μM against L1210 and 5.5 μM against HeLa tumor cells. All complexes were also tested for antiviral activity against a wide variety of DNA and RNA viruses, but found not to display selective activity at subtoxic concentrations.

Key words: Ruthenium complex, Cytotoxicity, Antiviral, Ligands, MLCT

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INTRODUCTION

Ongoing interests in these laboratories are focused on ruthenium for which various ruthenium complexes display cytotoxic properties (1-5). The review of literature revealed that the discovery of the anticancer properties of cisplatin in 1965 heralded the development of metallopharmaceuticals and caused a revolution in cancer therapy (6). Platinum drugs are believed to induce cytotoxicity by cross-linking DNA, causing changes to the DNA structure that inhibit replication and protein synthesis. However, the application of platinum drugs suffers from their high general toxicity leading to severe side effects. In comparison, ruthenium complexes have attracted considerable attention in the last 20 years as potential antitumor agents. Some of them, indeed, exhibit very encouraging pharmacological profiles (7). The advantage of Ru complexes compared to Pt complexes is their relative low toxicity (8,9). Recently, two new NAMI-A types of complexes containing hydroxamic acid \([\text{3-pyhaH}][\text{trans-RuCl}_4(\text{dmso-S})(3\text{-pyha})]\) and \([\text{4-pyhaH}][\text{trans-RuCl}_4(\text{dmso-S})(4\text{-pyha})]\) have been reported in the literature. It has been suggested that the increased production of gelatinases by treated tumor cells might be due to the release of NO from the hydroxamic acid moieties present in these complexes (10). Therefore, ruthenium complexes show great promise, not only because they offer reduced toxicity, compared with that of other metals, but also because their complexes should have different mechanisms of action and, consequently, a different spectrum of activity and no cross-resistance (11,12). It is recently discovered that Ruthenium polyaminocarboxylate (Ru-pac complexes) possess cysteine protease inhibitory activity. The discovery of the protease inhibitory activity of Ru-pac complexes may be of significance in developing antiviral agents in which Ru-pac complexes could act as metallo-inhibitor agents for disease progression (13). Three new complexes of the general formula \(L[RuCl_2(\text{DMso})]\), where \(L=\text{chlorpromazine hydrochloride, trifluoperazine dihydrochloride, thioridazine hydrochloride, were prepared and carried superoxide dismutase (SOD) and catalase (CAT) activity under physiological conditions (14). }

A new ligand and two ruthenium(II) complexes \([\text{Ru(bpy)}_2(\text{DNPIP})][\text{ClO}_4]\) and \([\text{Ru(bpy)}_2(\text{DAPIP})][\text{ClO}_4]\) \((\text{bpy}=2,2'\text{-bipyridine, DNPIP}=2-(2,4-dinitrophenyl) imidazo[4,5-f][1,10]phenanthroline) and DAPIP=2-(2,4-diaminophenyl)imidazo[4,5-f][1,10]phenanthroline) were synthesized and shown to bind to CT-DNA by an intercalative mode and induced apoptosis in BEL-7402 cell cultures (15). The study described by Heinrich et al. in 2011 (16), on the synthesis of a new ruthenium nitrosyl complex with the formula \([\text{RuCl}_2\text{-NO}(\text{BPA})]\) \([\text{BPA}=2\text{-hydroxybenzyl}(2\text{-methylpyridyl}) \text{amine ion}]\), performed \text{in vitro} cytotoxic assays, which revealed its cytotoxic activity against two different tumor cell lines (HeLa and Tm5), with efficacy comparable to that of cisplatin. The \text{in vivo} studies showed that \([\text{RuCl}_2\text{-NO}(\text{BPA})]\) is effective in reducing tumor mass. They showed that the mechanism of action of \([\text{RuCl}_2\text{-NO}(\text{BPA})]\) is by binding to DNA, causing fragmentation of this biological molecule, which leads to apoptosis. Tan et al. in 2011 (17), synthesized two new ruthenium complexes of the type \(\text{trans, cis-}[\text{RuCl}_2(\text{DMso})(\text{H}_2\text{biim})]\) [1] and \(\text{mer-[RuCl}_3(\text{DMso})(\text{H}_2\text{biim})]\) [2] (DMso=dimethyl sulfoxide and H2biim=2,2'-bimidazole), which were fully characterized by single-crystal X-ray analysis. The less stable complex [2] was more cytotoxic than [1] against the four human cancer cell lines tested. Further studies showed that [1] and [2] exhibited cell growth inhibition by triggering G0/G1 cell cycle arrest and mitochondria-mediated apoptosis. Additionally, complex [2] exerts potent inhibitory effects on the adhesion and migration of human cancer cells comparable to that of NAMI-A \(([\text{ImH}]\text{[trans-}[\text{RuCl}_4(\text{Im})(\text{DMSO-S})]], \text{Im}=\text{imidazole}).\) Ruthenium (II) arene complexes show remarkable cytotoxic properties \text{in vitro} as well as \text{in vivo} (18). A series of complexes with the general formula \([\text{Ru(n}_2\text{-arene})\text{Cl}(\text{en})][\text{PF}_6]\) \((\text{en}=\text{ethylenediamine, arene}=\text{benzene, p-cymene, tetrahydroanthracene etc}) have been studied for their \text{in vitro} anticancer activity (19).
Our research has been focused on complexes of general formula [Ru(L)₂L₁]Cl₂, where L=2,2'-bipyridine/1,10-phenanthroline/DMSO and L₁=HBT, FCi-HBT, IIH, NO₂-MPC, OCH₃-MPC, N(CH₃)₂-MPC or Cl-MPC.

EXPERIMENTAL

General methods

The solvents (AR grades) were obtained from Sd Fine Chem., Mumbai, and E. Merck, Mumbai. The reagents (puriss grade) were obtained from Fluka and E. Merck. Hydrated ruthenium trichloride was purchased from Loba Chemie, Mumbai, and used as received. UV–visible spectra were run on a Jasco spectrophotometer. FTIR spectra were recorded in KBr powder on a Jasco V410 FTIR spectrophotometer by diffuse reflectance technique. ¹H NMR spectra were measured in CDCl₃ and DMSO-d₆ on a Bruker Ultraspec AMX 400 MHz spectrometer. The reported chemical shifts were against that of TMS. TKA 5 was prepared according to literature (31).

Preparation of (4-substituted benzylidene)-isonicotinohydrazide (R-MPC) where R = NO₂, OCH₃, N(CH₃)₂, Cl (20)

In a round bottom flask take 0.007 mol of substituted benzaldehyde and equimolar quantity of (0.007 mol) isoniazide, 0.5 ml of glacial acetic acid and 50 ml of ethanol. The reaction mixture was refluxed for four to five hours in a water bath. The reaction mixture was cooled and the precipitated mass was filtered. It was then recrystallized from ethanol and dried at room temperature.

N’-[(4-nitrophenyl)methylidene]pyridine-4-carbohydrazide (NO₂-MPC)

Yield 92 %, M P: 235-238 °C, IR: 3437, 3205, 3038, 2872, 1668, 1580, 1356, 1300. ¹H NMR: 12.11 (1H, s), 8.79 (d, 2H, J=8 Hz), 8.45 (s, 1H), 7.82 (d, 2H, J=8 Hz), 7.78 (d, 2H, J=8 Hz), 7.54 (d, 2H, J=8 Hz).

N’-[(4-methoxyphenyl)methylidene]pyridine-4-carbohydrazide (OCH₃-MPC)

Yield 96 %, M P: 120-123 °C, IR: 3444, 3116, 3040, 2876, 1658, 1604, 1310, 1254. ¹H NMR: 12.10 (1H, s), 8.75 (d, 2H, J=8 Hz), 8.43 (s, 1H), 7.80 (d, 2H, J=8 Hz), 7.82 (d, 2H, J=8 Hz), 7.57 (d, 2H, J=8 Hz), 3.88 (s, 3H, OCH₃).

N’-[(4-(dimethylamino)phenyl)methylidene]pyridine-4-carbohydrazide (N(CH₃)₂-MPC)

Yield 95 %, M P: 205-208 °C, IR: 3119, 3032, 2923, 1690, 1576, 1536, 1298. ¹H NMR: 12.13 (1H, s), 8.80 (d, 2H, J=8 Hz), 8.43 (s, 1H), 7.84 (d, 2H, J=8 Hz), 7.79 (d, 2H, J=8 Hz), 7.55 (d, 2H, J=8 Hz), 2.91 (s, 6H, N(CH₃)₂).

Preparation of (N’-[2-oxo-1,2-dihydro-3H-indol-3-ylidene]pyridine-4-carbohydrazide (IINH) (21)

In a round bottom flask was put 0.004 mol of isatin and an equimolar quantity of (0.004 mol) isoniazide, 0.5 ml of glacial acetic acid and 50 ml of ethanol. The reaction mixture was refluxed for four to five hours in a water bath. The reaction mixture was cooled and the precipitated mass was filtered. It was then recrystallized from ethanol and dried at room temperature.

Yield: 68 %, M P: 295-298 °C, IR: 3344, 3097, 1640, 1561, 1434, 1281, 1148. ¹H NMR: 13.95 (s, 1H), 11.39 (s, 1H), 8.86 (d, 2H, J=8 Hz), 7.78 (d, 2H, J=8 Hz), 7.60 (s, 1H, ar), 7.41 (t, 1H, J=16 Hz), 7.11 (t, 1H, J=16 Hz), 6.91 (d, 1H, J=8 Hz).

Preparation of 2-hydrazinyl-1,3-benzothiazole (HBT) (22)

In a round bottom flask a suspension of 7.5 g of 2-aminobenzothiazole (BT) (23) 40 ml of ethylene glycol, 10 ml of (99%) hydrazine hydrate and 10 ml of concentrated hydrochloric acid was added at 5-6 °C. The reaction mixture was refluxed for 2-3 hr and cooled to room temperature. The reaction mixture was filtered and the resulting precipitate was washed with distilled water. The resulting crude product was crystallized...

Preparation of 5-chloro-6-fluoro-2-hydrazinyl-1,3-benzothiazole FC1-HBT (24)

1st step: Synthesis of 5-chloro-6-fluoro-1,3-benzothiazol-2-amine

To glacial acetic acid (20 ml) precooled to 0-5 °C were added 20 g of potassiumthiocyanate and 3.6 g (0.025 mol) of 4-chloro-3-fluoroaniline. The mixture was placed in freezing mixture of ice and salt and mechanically stirred while 3 ml of bromine in 12 ml of glacial acetic acid was added from a dropping funnel at such a rate that the temperature did not rise beyond 0 °C. After all the bromine has been added (105 min), the solution was stirred for an additional 2 h at 0 °C and at room temperature for 10 h. It was allowed to stand overnight during which an orange precipitate settled at the bottom, water (30 ml) was added quickly and slurry was heated at 85 °C in a steam bath and filtered while hot. The orange residue was placed in a reaction flask and treated with 5 ml of glacial acetic acid, heated again to 85 °C in a steam bath and filtered while hot. The orange residue was placed in a reaction flask and treated with 5 ml of glacial acetic acid, heated again to 85 °C and filtered when hot. The combined filtrate was cooled and neutralized with concentrated ammonia solution to pH 6 when a dark yellow precipitate was collected, which was recrystallized from an ethanol-water mixture. Yield 92 %, M P: 185-188 °C, IR: 3418, 3250, 3065, 1596, 1556, 1443, 1287, 1146.

2nd step: In a round bottom flask a suspension of 5 g of 5-chloro-6-fluoro-1,3-benzothiazol-2-amine in 40 ml of ethylene glycol, 12 ml of (99 %) hydrazine hydrate and 12 ml of concentrated hydrochloric acid was added at 5-6 °C. The reaction mixture was refluxed for 2-3 h and cooled to room temperature. The reaction mixture was filtered and the resulting precipitate was washed with distilled water. The resulting crude product was recrystallized from ethanol. Yield 65 %, M P: 242-245 °C, IR: 3478, 3402, 3095, 1651, 1550, 1456, 1194.

Method for the Preparation of [Ru(DMSO)Cl2] (25)

1 g of ruthenium trichloride trihydrate and 5 ml of dimethyl sulphoxide (DMSO) was taken in a round bottom flask and refluxed for 5 min. The volume was reduced to half, when addition of (20 ml) acetone gave a yellow precipitate. The yellow complex which separated was filtered off, washed with acetone and ether, and vacuum dried. The crude yellow precipitate was recrystallized from dimethyl sulphoxide yielding yellow crystals. [M.P: 193-195 °C].

General Method for the Preparation of [Ru(DMSO)2(L)Cl2] L = HBT, FC1-HBT or IINH

In a round bottom flask taken 0.31 mmol of Ru(DMSO)Cl2 and equimolar quantity of ligand L and 40 ml of toluene, were refluxed for 40 min. The volume was reduced to 5 ml and ether was added slowly, with vigorous stirring the precipitate was obtained and filtered off. The crude precipitate was recrystallized from a suitable solvent.

[Ru(dmso)2(2-hydrazinyl-1,3-benzothiazole)Cl2] (RDB-1)

Yield 47 %, IR: 3442, 3293, 3063, 2927, 1590, 1507, 1443, 1254. 1H NMR: 7.32-6.97 (4H, m, ar), 2.52 (12H, 4 CH3, alkyl). +ESI m/z: 493.5 (M).

[Ru(dmso)2(5-chloro-6-fluoro-2-hydrazinyl-1,3-benzothiazole)Cl2] (RDB-3)

Yield 45 %, IR: 3471, 3157, 2927, 1627, 1535, 1457, 1275, 1227, 1079. +ESI m/z: 545.9 (M).

[Ru(dmso)2(N’-[2-oxo-1,2-dihydro-3H-indol-3-ylidene]pyridine-4-carbohydrazide)Cl2] (RDB-4)

Yield 40 %, IR: 3340, 3191, 2961, 2925, 1616, 1459, 1081. +ESI m/z: 594.5 (M).

Preparation of cis-[bis(L)dichloro-ruthenium(II)]cis-[Ru(L)2Cl2] (where L=2,2’-bipyridine/1,10-phenanthroline) (26)

RuCl3.xH2O 1.15 g (2.5 mmol) and ligand L (5 mmol) were refluxed in 50 ml DMF for 3 h under nitrogen atmosphere. The reddish brown solution slowly turned purple and the product precipitated in the reaction mixture. The solution was cooled overnight at 0 °C. A fine microcrystalline mass was filtered off. The residue was repeatedly washed with 30 % LiCl solution and finally recrystallized. The
product was dried and stored in a vacuum desiccator over P$_2$O$_5$ for further use (75 %).

**General procedure for preparing [Ru(L)$_2$(L1)]Cl$_2$ (where L=1,10-phenanthroline /2,2'-bipyridine and where L1 = NO$_2$-MPC, OCH$_3$-MPC, N(CH$_3$)$_2$-MPC or Cl-MPC)***

To the black microcrystalline cis-bis(L)dichloro ruthenium(II) / cis-Ru(L)$_2$Cl$_2$ (2 mmol), an excess of ligand L1 (2.5 mmol) was added and refluxed in ethanol under nitrogen atmosphere. The initial colored solution slowly changed to a brownish orange at the end of the reaction, which was verified by TLC on silica plates. Finally, they were purified by column chromatography using silica gel as stationary phase and chloroform–methanol as mobile phase.

**[Ru(bpy)$_2$(N'-[(4-nitrophenyl)methylidene]pyridine-4-carbohydrazide)]Cl$_2$ (TKA-3)**

Yield 45 %, IR: 3476, 3070, 2967, 2863, 1675, 1530, 1532, 1288, 1155. NMR δ: 7.30 (t, 1H, J=16 Hz), 7.36 (t, 1H, J=16 Hz), 7.58 (d, 1H, J=5.2 Hz), 7.79-7.73 (m, 4H, ar), 7.95-7.73 (m, 4H, ar), 8.31-8.15 (m, 5H, ar), 8.27 (br, s, 1H, NH), 8.53 (d, 1H, J=4.8 Hz), 8.60 (d, 1H, J=8 Hz), 8.69-8.62 (m, 3H, ar), 8.76-8.74 (d, 1H, J=8 Hz), 8.80 (d, 1H, J=8 Hz), 9.86 (d, 1H, J=8 Hz), 11.87 (s, 1H, =CH-). LCMS: Ru(bpy)$_2$(NO$_2$-MPC)Cl: m/z, calcd 719.1, found 719.1.

**[Ru(bpy)$_2$(OCH$_3$-MPC)]Cl$_2$ (TKA-4)**

Yield 48 %, IR: 3473, 3068, 2962, 2925, 1673, 1602, 1535, 1454, 1414, 1290, 1153, 766. NMR δ: 7.30 (t, 1H, J=8 Hz), 7.36 (t, 1H, J=8 Hz), 7.53 (d, 2H, J=8 Hz), 7.58 (d, 1H, J=5.2 Hz), 7.78-7.71 (m, 5H, ar), 7.86 (d, 1H, J=8 Hz), 7.95-7.89 (m, 4H, ar), 8.21-8.15 (m, 2H, ar), 8.44 (s, 1H, ar), 8.53 (d, 1H, J=8 Hz), 8.60 (d, 2H, J=8 Hz), 8.69-8.66 (m, 2H, ar), 8.81 (d, 1H, J=8 Hz), 9.85 (d, 1H, J=5.2 Hz), 12.26 (s, 1H, =CH-). LCMS: Ru(bpy)$_2$(OCH$_3$-MPC)Cl: m/z, calcd 708.53, found 708.0.

**[Ru(bpy)$_2$(N'(4-chlorophenyl)methylidene]pyridine-4-carbohydrazide)]Cl$_2$ (TKA-6)**

Yield 48 %, IR: 3471, 3069, 2960, 2865, 1677, 1533, 1354, 1295, 1168. NMR δ: 7.30 (t, 1H, J=8 Hz), 7.36 (t, 1H, J=8 Hz), 7.53 (d, 2H, J=8 Hz), 7.58 (d, 1H, J=5.2 Hz), 7.78-7.71 (m, 5H, ar), 7.86 (d, 1H, J=8 Hz), 7.95-7.89 (m, 4H, ar), 8.21-8.15 (m, 2H, ar), 8.44 (s, 1H, ar), 8.53 (d, 1H, J=8 Hz), 8.60 (d, 2H, J=8 Hz), 8.69-8.66 (m, 2H, ar), 8.81 (d, 1H, J=8 Hz), 9.85 (d, 1H, J=5.2 Hz), 12.26 (s, 1H, =CH-). LCMS: Ru(bpy)$_2$(Cl-MPC)Cl: m/z, calcd 766.5, found 767.

**[Ru(phen)$_2$(N'-[(4-nitrophenyl)methylidene]pyridine-4-carbohydrazide)]Cl$_2$ (TKA-7)**

Yield 44 %, IR: 3471, 3069, 2960, 2865, 1677, 1533, 1354, 1295, 1168. NMR δ: 7.58-7.48 (m, 2H, ar), 7.78-7.72 (m, 4H, ar), 8.16-8.12 (m, 3H, ar), 8.20 (d, 2H, J=8 Hz), 8.25 (d, 2H, J=8 Hz), 8.34-8.32 (m, 2H, ar), 8.39 (d, 1H, J=8 Hz), 8.48 (d, 2H, J=8 Hz), 8.52-8.50 (m, 2H, ar), 8.90-8.79 (m, 3H, ar), 9.06 (d, 1H, J=8 Hz), 10.20 (s, 1H), 12.39 (s, 1H, =CH-). LCMS: Ru(phen)$_2$(NO$_2$-MPC)Cl: m/z, calcd 766.5, found 767.

**[Ru(phen)$_2$(4-(4-methoxyphenyl)methylidene]pyridine-4-carbohydrazide)]Cl$_2$ (TKA-8)**

Yield 50 %, IR: 3475, 3066, 2845, 1670, 1610, 1560, 1455, 1255, 1165. NMR δ: 3.30 (s, 3H, OCH$_3$), 7.01 (d, 2H, J=8 Hz), 7.51-7.48 (dd, 1H, J=8.8 Hz), 7.57-7.54 (dd, 1H, J=8, 8 Hz), 7.64 (d, 2H, J=8 Hz), 7.69 (d, 2H, J=8 Hz), 7.75-7.73 (m, 1H, ar), 8.16-8.11 (m, 2H, ar), 8.23-8.16 (m, 2H, ar), 8.29-8.24 (m, 1H, ar), 8.34-8.30 (m, 3H, ar), 8.39-8.37 (m, 1H, ar), 8.48 (t, 2H, J=16), 8.86-8.83 (m, 3H,
ar), 9.06-9.05 (m, 1H, NH), 10.20 (s, 1H, NH), 11.94 (s, 1H, =CH-). LCMS: Ru(phen)$_2$(OCH$_3$-MPC): m/z, calcd 716.0, found 716.0.

[Ru(phen)$_2$(N'-[(4-(dimethylamino)phenyl)methylidene]pyridine-4-carbohydrazide)]Cl$_2$ (TKA-9)
Yield 52 %, IR: 3469, 3070, 2920, 2861, 1659, 1601, 1529, 1455, 1300, 1184. NMR δ: 2.95 (s, 6H, -N(CH$_3$)$_2$), 7.51-7.48 (m, 2H, ar), 7.58-7.53 (m, 2H, ar), 7.68-7.67 (m, 2H, ar), 7.75 (d, 1H, J=8 Hz), 7.82-7.80 (m, 1H, ar), 8.16-8.11 (m, 2H, ar), 8.29-8.18 (m, 4H, ar), 8.34-8.30 (m, 2H, ar), 8.39 (d, 1H, J=8 Hz), 8.48 (t, 2H, J=16 Hz), 8.86-8.83 (m, 4H, ar), 9.06 (d, 1H, J=8 Hz), 10.20-10.18 (s, 1H, NH), 11.77 (s, 1H, =CH-). LCMS: Ru(phen)$_2$(N(CH$_3$)$_2$-MPC): m/z, calcd 729.0, found 729.1.

[Ru(phen)$_2$(N'-[(4-chlorophenyl)methylidene]pyridine-4-carbohydrazide)]Cl$_2$ (TKA-10)
Yield 52 %, IR: 3469, 3071, 2965, 2928, 1671, 1600, 1551, 1459, 1418, 1290, 1152, 777. NMR δ: 7.51-7.48 (m, 3H, ar), 7.58-7.54 (m, 2H, ar), 7.77-7.70 (m, 4H, ar), 8.15-8.09 (m, 2H, ar), 8.22 (d, 1H, J=8 Hz), 8.25 (d, 1H, J=8 Hz), 8.34-8.30 (m, 2H, ar), 8.43-8.37 (m, 2H, ar), 8.48 (t, 2H, J=16 Hz), 8.79 (d, 1H, J=8 Hz), 8.86-8.83 (m, 3H, ar), 9.06-9.05 (m, 1H, ar), 10.20 (s, 1H, =NH-), 12.18 (s, 1H, =CH-). LCMS: Ru(phen)$_2$(Cl-MPC): m/z, calcd 720.69, found 720.1.

Biological assays
The antiviral assays were based on the inhibition of virus-induced cytopathicity in confluent cell cultures, and the cytostatic assays on inhibition of tumor cell proliferation in exponentially growing tumor cell cultures.

Cytotoxic and antiviral activity assays
The antiviral assays (27) were based on inhibition of virus-induced cytopathicity in HEL [herpes simplex virus type 1 (HSV-1) (KOS), HSV-2 (G), vaccinia virus, vesicular stomatitis virus], Vero (parainfluenza-3, reovirus-1, sindbis virus and Coxsackie B4) and HeLa (vesicular stomatitis virus, Coxsackie virus B4, and respiratory syncytial virus) cell cultures. Confluent cell cultures in microtiter 96-well plates were inoculated with 100 CCID$_{50}$ of virus (1 CCID$_{50}$ being the virus dose to infect 50% of the cell cultures) and incubated in the presence of varying concentrations (200, 40, 8, ... µg/mL) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. The minimal cytotoxic concentration (MCC) of the compounds was defined as the compound concentration that caused a microscopically visible alteration of cell morphology.

Cytostatic activity assays
The methodology for cytostatic activity assays in HeLa, CEM and L1210 cell cultures has been published previously (28). Murine leukemia L1210, human lymphocyte CEM and human epithelial cervical carcinoma HeLa cells were seeded in 96-well microtiter plates at 50,000 (L1210) or 75,000 (CEM, HeLa) cells per 200 µL-well in the presence of different concentrations of the test compounds. After 2 (L1210) or 3 (CEM, HeLa) days, the viable cell number was counted using a Coulter counter apparatus. The 50% cytostatic concentration (CC$_{50}$) was defined as the compound concentration required to inhibit tumor cell proliferation by 50%.

Effects of Binding to DNA on Visible MLCT Transitions (29,30)
All the experiments involving the interaction of the complexes with DNA were carried out using 5mM Tris buffer at pH 7.4 with 50mM NaCl. Keeping the concentration of the complexes constant (2.5m M) and varying the concentration of DNA (20-50m g/ml), the absorption titration was carried out. Changes in the MLCT band of the complexes were noted. The shifts in the MLCT bands of the complexes were reported. The shifts in the MLCT bands at the highest concentration of DNA are reported.

RESULTS AND DISCUSSION
Chemistry
Results are summarized in Tables 1 and 2 and Schemes 1, 2 and 3 show the details of
the synthetic strategy adopted for the synthesis of ligands and homoleptic ruthenium complex. Ruthenium trichloride trihydrate undergoes reduction in a number of organic solvents. In this homoleptic chelate the first ligand to enter the complex in a stepwise assembly were 2,2'-bipyridine / 1,10-phenathroline respectively. A single step method was adopted for first ligand synthesis. Ruthenium trichloride trihydrate was refluxed in DMF in the presence of 2,2'-bipyridine / 1,10-phenathroline, in excess of the stoichiometric amount, which afforded the final product cis-bis(2,2'-bipyridine / 1,10-phenathroline)-dichlororuthenium(II) (Scheme 2). The introduction of the third ligand was carried out in the presence of alcohol for TKA-3 to TKA-10 (Scheme 3). Ruthenium trichloride trihydrate was refluxed in DMSO, in which the final product obtained was dichlorotetrakis-(dimethyl sulphoxide) ruthenium (II) (Scheme 1). The final chelate formed had ionic chloride in the molecule. The RDB-1, RDB-3, RDB-4 was prepared by refluxing dichlorotetrakis-(dimethyl sulphoxide) ruthenium (II) with respective ligand in toluene (Scheme 1).

All these ligands were confirmed for their purity by their melting point, IR-spectra. In ligand 2-hydrazinyl-1,3-benzothiazole (HBT) the vibration bands were at 3479 for NH₂, 3369 for NH, 3174 for CH (aromatic). In 5-chloro-6-fluoro-2-hydrazinyl-1,3 -benzothiazole (FCl-HBT) ligand the vibration bands were exhibited at 3478 for NH₂, 3402 for NH, 3095 for CH (aromatic). In IINH ligand IR vibration bands were exhibited at 3344 for NH, 3097 for CH (aromatic), 1640 for C=O. In ¹H-NMR of IINH, there are well resolved resonance peak at low field at 13.94 (1H, s, NH), 11.39 (1H, s, NH) and 8.86-6.91 (8H, Ar-H) 5 ppm, respectively. In R-MPC ligands the IR bands exhibited their vibration

The ligand IINH was prepared by heating isatin with isoniazid in alcohol (21). Substituted benzyl isonicotinohydrazide (R-MPC) were prepared by refluxing, respective benzaldehyde with isonicotinic acid in good yields (20).

All these ligands for high purity, it was necessary to use column chromatography. Column chromatography was performed with silica gel (60-120 mesh) as the support with CHCl₃ / CHCl₃-CH₃OH as the eluate.
bands from 3463-3411 for NH, 3188-3038 for CH (aromatic), 2976-2852 for CH (aliphatic), 1668-1658 for C=O stretching (Fig 1).

The IR spectra of complex (RDB-1) \([\text{Ru(DMSO)}_2(\text{HBT})]\text{Cl}_2\) the IR bands observed at 3442 for NH, 3293 for NH, 3063 for CH (aromatic), 2927 for CH (aliphatic). In (RDB-4) \([\text{Ru(dmso)}_2(\text{IINH})]\text{Cl}_2\) complex the IR vibration bands were seen at 3340 for NH, 4-OCH$_3$, 4-N(CH$_3$)$_2$, 4-Cl) that no longer has a C2 axis of symmetry, resulting in non equivalency of ligands. Such a loss of C2 axis of symmetry and resulting to non equivalency of ligands has been observed in literature (1-5). Therefore such NMR spectra will be more complicated. In $^1$H-NMR spectra of complexes there are well resolved resonance peak at low field at 12.50-11.77 for -CO-NH-, and from 10.20-9.85 for -N=CH-, 9.06-6.75 for aromatic hydrogens and for -OCH$_3$ at 3.30 ppm (TKA-4 & 8), for -N(CH$_3$)$_2$ at 2.96 ppm (TKA-5 & 9), respectively.

These complexes showed broad and intense visible bands between 350 and 450 nm due to metal to ligand charge transfer transition. In the UV region, the bands at 290 and 310 nm were assigned to the phenanthroline ligand. The same transition was found in free phenanthroline at 280 nm, so that coordination of the ligand results in a red shift in the transition energy. There were also two shoulders at 390 and 500 nm, which were tentatively attributed to a metal to ligand charge transfer transitions involving phenanthroline ligand.

The LC-MS of the prepared complexes showed mass spectra for their respective masses. Thus, based on the above observations, the proposed structures of the

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{ligands.png}
\caption{Structures of the ligands (IINH, HBT, 2,2'-bipyridine, RMPC, FCl-HBT and 1,10-phenanthroline).}
\end{figure}
complexes are octahedral coordination and showed in Figure 2.

**Biological activity and discussion**

The *in vitro* cytostatic activity was evaluated for the ruthenium complexes and the results are summarized in Table 1. The cytostatic data (Table 1) revealed that several ruthenium complexes have antiproliferative potencies. Of the tested ruthenium complexes, TKA-9 showed pronounced cytostatic activity against all three cell lines tested. Its IC\textsubscript{50} ranked in the range of 5.5 to 20 µM, which is much more pronounced than observed for the other ruthenium complexes. The 1,10-phenanthrolines show, in general, somewhat higher inhibitory activity against tumor cell proliferation than the 2,2'-bipyridines. It is currently unclear why TKA-9 is superior to the other derivatives regarding cytostatic activity. There is a tendency that the human tumor cell lines were somewhat more sensitive to the anti-proliferative activity of the ruthenium complexes than the murine tumor cell line. However, in many cases, the compounds did not significantly affect the tumor cell proliferation at 250 µM. The compounds have also been evaluated for their inhibitory activity against a wide variety of DNA and RNA viruses (see experimental procedures) and the antiviral activity data (Table 2) revealed that ruthenium complexes RDB-3, TKA-3 and TKA-6 showed very modest activity against vesicular stomatitis virus and Coxsackie virus B4 in HeLa cell cultures. The fact that these complexes proved inactive against VSV-infected HEL and Coxsackie virus B4-infected Vero cell cultures let us to conclude that there is most likely not a specific antiviral effect of these compounds. The slight anti-VSV and Coxsackie virus B4 activity might be due to underlying toxicity of the complexes. A study on DNA binding of the synthesized complex (TKA3) was performed and it did not show any interaction with calf thymus DNA as there was no shift in the visible MLCT (metal to ligand charge transfer) bands (29).
CONCLUSION

In conclusion, eleven ruthenium (RDB-1, RDB-3, RDB-4 and TKA-3 to TKA-10) complexes, bearing 2,2'-bipyridine, 1,10-phenanthroline and dimethylsulfoxide with HBT, FCI-HBT, IINH, NO\(_2\)-MPC, OCH\(_3\)-MPC, N(CH\(_3\))\(_2\)-MPC & Cl-MPC were synthesized in alcohol. The coordination involved for TKA-3 to TKA-10 complexes is via C=O and imine nitrogen. From the antiviral and cytostatic results presented in Tables 1 and 2, it is clear that ruthenium complex TKA-9 exhibited an inhibitory effect on the proliferation of tumor cells (IC\(_{50}\) as low as 5.5 µM to 20 µM). Its mechanism of action is currently unclear.

ACKNOWLEDGEMENTS

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Table 1. Inhibitory effects of Ruthenium complexes on the proliferation of murine leukemia cells (L1210), human T-lymphocyte cells (CEM) and human cervix carcinoma cells (HeLa)

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC(_{50}) (µM)</th>
<th>L1210</th>
<th>CEM</th>
<th>HeLa</th>
</tr>
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<tbody>
<tr>
<td>NO(_2)-MPC</td>
<td>≥ 250</td>
<td>173</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>OCH(_3)-MPC</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
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<tr>
<td>N(CH(_3))(_2)-MPC</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
</tr>
<tr>
<td>Cl-MPC</td>
<td>&gt; 250</td>
<td>137±9</td>
<td>135±23</td>
<td></td>
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<tr>
<td>HBT</td>
<td>184±2</td>
<td>137±9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCI-HBT</td>
<td>102±10</td>
<td>42±11</td>
<td>90±27</td>
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<tr>
<td>IINH</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
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<tr>
<td>RDB-1</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
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<tr>
<td>RDB-3</td>
<td>243 ± 11</td>
<td>147 ± 47</td>
<td>100 ± 9</td>
<td></td>
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<tr>
<td>RDB-4</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
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<tr>
<td>TKA-3</td>
<td>&gt; 250</td>
<td>167 ± 72</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
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<td>TKA-4</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
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<td>TKA-5</td>
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<td>172 ± 88</td>
<td>105 ± 32</td>
<td>&gt; 100</td>
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<tr>
<td>TKA-6</td>
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<td>&gt; 250</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
</tr>
<tr>
<td>TKA-7</td>
<td>&gt; 250</td>
<td>216 ± 56</td>
<td>160 ± 63</td>
<td>&gt; 100</td>
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<tr>
<td>TKA-8</td>
<td>147 ± 4</td>
<td>144 ± 52</td>
<td>94 ± 5</td>
<td></td>
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<tr>
<td>TKA-9</td>
<td>20 ± 2</td>
<td>16 ± 9</td>
<td>5.5 ± 0.2</td>
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<tr>
<td>TKA-10</td>
<td>108 ± 4</td>
<td>115 ± 40</td>
<td>102 ± 6</td>
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</table>

*p50% inhibitory concentration

Table 2. Cytotoxicity and antiviral activity of compounds in HeLa cell cultures

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration unit</th>
<th>Cytotoxicity</th>
<th>Antiviral Activity (EC(_{50}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CC(_{50}) (HeLa)</td>
<td>Minimum cytotoxic concentration (HeLa)</td>
</tr>
<tr>
<td>RDB-3</td>
<td>µM</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>TKA-3</td>
<td>µM</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>TKA-6</td>
<td>µM</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>DS-5000</td>
<td>µg/ml</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Ribavirin</td>
<td>µM</td>
<td>&gt;250</td>
<td>&gt;250</td>
</tr>
</tbody>
</table>

\(a\)50% Cytotoxic concentration, as determined by measuring the HeLa cell viability with the colorimetric formazan-based MTS assay.

\(b\)Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.

\(c\)50% Effective concentration, or compound concentration affording 50% inhibition of the virus-induced cytopathic effect (CPE), as determined by visual scoring of the cytopathicity, or by measuring the cell viability with the colorimetric formazan-based MTS assay.

Data are the mean of 2 independent experiments.
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