Turk J. Pharm. Sci. 8 (3), 207-218, 2011

Original article

SYNTHESIS, CHARACTERIZATION AND CYTOTOXIC ACTIVITY OF SOME Ru(II) COMPLEXES

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Abstract

The synthesis and characterization of ruthenium complexes (Ru1–Ru8) of the type [Ru(S)$_2$(K)], (where S = 1,10-phenanthroline/ 2,2'-bipyridine and K = iinh, inhba, na, mitsz) are described. These ligands form bidentate octahedral ruthenium complexes. The title compounds were subjected to in vitro cytostatic activity measurements against the human cancer T-lymphocyte cell lines Molt 4/C8 and CEM, and the murine tumor leukemia cell line L1210. In vitro evaluation of these ruthenium compounds revealed cytotoxic activity from 0.84 to 119 µg/mL against CEM, 3.7 to 158 µg/mL against Molt and 15 to ≥200 µg/mL against L1210 cell proliferation, depending the nature of the compound.

Key words: *Ruthenium complexes, Cytotoxicity, MLCT.*

Bazı Ru(II) Komplekslerinin Sentezi, Karakterizasyonu ve Sitotoksik Aktiviteleri

Bu çalışmada [Ru(S)$_2$(K)] (S = 1,10-fenantrolin/2,2'-bipiridin ve K = iinh, inhba, na, mitsz) tipi rutenium komplekslerinin (Ru 1 – Ru8) sentezi ve karakterizasyonu tanımlanmıştır. Bu ligandlar bidentate oktahedral rutenium kompleksini oluştururlar. Bu bileşiklerin sitotoksik aktiviteleri T-lenfosit (Molt 4/C8 ve CEM) kanser hücresi hatlarında ve lösemi (L1210) hücre hatlarında ölçülmüştür. İncelenen rutenium komponentlerinin in-vitro değerlendirmeleri sonucunda CEM için 0.84 den 119 µg/mL, Molt için 3,7 den 158 µg/mL ve L1210 için ise 15 den ≥200 µg/mL kadar komponentlerin yapısına bağlı olarak değişen hücre proliferasyonu tespit edilmiştir.

Anahtar kelimeler: *Rutenium kompleksleri, Sitotoksisite, MLCT.*

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INTRODUCTION

Various ruthenium complexes having the general formula $[Ru(S)_2(K)]$, [where S = 1,10-phenathroline/2,2'-bipyridine and K= itsz, MeO-btsz, hfc, 4-Cl-btsz etc. itsz=isatin-3-thiosemicarbazone, MeO-btsz= 1-(4'-methoxybenzyl)-thiosemicarbazone, hfc=2-{[3-chloro-4-fluoro-phenylimino]methyl}phenol, 4-Cl-btsz= 1-(4'-chlorobenzyl)thiosem-icarbazone)] and display cytotoxic properties (1).

The objectives of the present investigations were to develop analogs of $[Ru(S)_2(K)]$ principally as candidate cytotoxins. The review of literature revealed that the discovery of the anticancer properties of cisplatin (2) in 1965 marked the development of metallopharmaceuticals and heralded a revolution in cancer chemotherapy. Platinum drugs are believed to induce cytotoxicity by cross-linking DNA, causing changes to the DNA structure resulting in inhibition of replication and protein synthesis. However, the application of platinum drugs suffers from their high general toxicity leading to severe toxic side-effects. In comparison, ruthenium complexes have attracted large attention in the last 20 years as potential antitumor agents. Some of them exhibit very encouraging pharmacological profiles (3).

The most prominent examples of ruthenium complexes so far are the Ru(III) complexes, $Na[trans\text{-}RuCl_4(DMSO)(imid)]$, NAMI, its more stable imidazolium analogue $[imidH][trans\text{-}RuCl_4(DMSO)(imid)]$, NAMI-A and the indazole complex $[indH][trans\text{-}RuCl_4(ind)_2]$, KP1019. Complex NAMI-A shows high selectivity for tumor metastasis (4,5) and low toxicity at pharmacologically active doses (6-8) and has successfully completed phase I clinical trials (9), complex KP1019 has also successfully completed phase 1 clinical trials (10).

Ruthenium (II) arene complexes show remarkable cytotoxic properties in vitro as well as in vivo (11-13). A series of complexes with the general formula $[Ru(\eta^6\text{-arene})Cl(en)][PF_6]$ (en=ethylenediamine, arene=benzene, p-cymene, tetrahydroanthrace-ne etc) have been studied for their in vitro anticancer activity (14).

Our research has been focused on synthesis, characterization and cytotoxic study of some ruthenium (II) complexes of the type $[Ru(S)_2(K)]Cl_2/Cl$ (where S = 2,2'-bipyridine/ 1,10-phenanthroline and K = iinh, inhba, na, mitsz).

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. Nalidixic acid was used as received from Microlabs, India. UV–visible spectra were run on a Jasco spectrophotometer. The ligands inhba (15, 16), iinh (17), and mitsz (18) were prepared according to the literature. FTIR spectra were recorded in KBr powder on a Jasco V410 FTIR spectrometer by diffuse reflectance technique. ^1H NMR spectra were measured in $CDCl_3$ and DMSO-d_6 on a Bruker Ultraspec AMX 400 MHz spectrometer. The reported chemical shifts were against that of TMS.

Preparation of cis-[bis(S)dichlororuthenium(II)]cis-$[Ru(S)_2Cl_2]$[19] (where S = 2,2'-bipyridine/ 1,10-phenanthroline)(19)

$RuCl_3.xH_2O$ 1.15 g (2.5 mmol) and ligand S (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_2O_5 for further use (75%).

General procedure for preparing [Ru(S)$_2$(K)]Cl$_2$ (where S = 1,10-phenanthroline /2,2'-bipyridine; where K = iinh, inhba, na, mitsz)

To the black microcrystalline cis-bis(S)dichloro ruthenium(II) cis-Ru(S)$_2$Cl$_2$/(2 mmol), excess of ligand K (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.

Ru1. [Ru(phen)$_2$(iinh)]Cl$_2$.
Yield 45%. FTIR (KBr) cm^{-1}: 3397 (N–H), 3045 (C–H), 1689, 1618 (C=O). λ_{max} nm (MeOH): 223, 258, 401, and 475. Anal. Calcd for $RuC_{38}H_{26}Cl_2N_8O_2$: C, 57.15; H, 3.28; N, 14.03. Found: C, 57.10; H, 3.21; N, 14.05. ^{1}H NMR (DMSO-d6): δ = 13.85 (1H, s, br), 11.39 (1H, s), 10.19 (1H, d, *J* = 4.4 Hz), 9.10 (1H, d, *J* = 5.0 Hz), 8.98 (2H, m), 8.87 (3H, d, *J* = 8.2 Hz), 8.51 (2H, t, *J* = 8.2 Hz), 8.42–8.39 (1H, m), 8.36–8.32 (2H, m), 8.29–8.22 (2H, m), 8.18–8.15 (1H, dd, *J* = 13.44, 8.22 Hz), 8.12 (1H, d, *J* = 4.8 Hz), 7.73 (1H, d, *J* = 4.9 Hz), 7.68 (2H, d, *J* = 6.28 Hz), 7.59-7.56 (1H, dd, *J* = 13.53, 8.16 Hz), 7.54-7.50 (1H, dd, *J* = 13.5, 8.2 Hz), 7.44-7.40 (1H, m), 7.14-7.11 (1H, t, *J* = 15 Hz), 6.96 (1H, d, *J* = 7.8 Hz).

Ru2. [Ru(bpy)$_2$(iinh)]Cl$_2$.
Yield 45%. FTIR (KBr) cm^{-1}: 3371 (N–H), 3049 (C–H), 1691 1615 (C=O). λ_{max} nm (MeOH): 223, 266, 399, and 472. Anal. Calcd for $RuC_{34}H_{26}N_8O_2Cl_2$: C, 54.41; H, 3.49; N, 14.93. Found: C, 54.39; H, 3.50; N, 14.92.

Ru3. [Ru(phen)$_2$(inhba)]Cl$_2$.
Yield 45%. FTIR (KBr) cm^{-1}: 3210 (N–H), 3040 (C–H), 1673 (C=O), 1609 (N–H). λ_{max} nm (MeOH): 221, 263, 405, and 472. Anal. Calcd for $RuC_{37}H_{27}N_7OCl_2$: C, 58.66; H, 3.59; N, 12.94. Found: C, 58.59; H, 3.53; N, 13.05. ^{1}H NMR (DMSO-d6): δ = 12.00 (1H, s, br), 10.21 (1H, d, *J* = 5.1 Hz), 9.09 (1H, d, *J* = 4.9 Hz), 8.88-8.81 (5H, m), 8.53-8.48 (2H, m), 8.42–8.40 (1H, m), 8.35–8.33 (3H, m), 8.29–8.27 (1H, m), 8.24–8.22 (1H, m), 8.19-8.14 (2H, m), 7.77 (1H, d, *J* = 4.6 Hz), 7.72-7.68 (3H, m), 7.60-7.56 (1H, dd, *J* = 8.2, 8.1 Hz), 7.54-7.50 (1H, m), 7.47-7.46 (3H, m).

Ru4. [Ru(bpy)$_2$(inhba)]Cl$_2$.
Yield 45%. FTIR (KBr) cm^{-1}: 3212 (N–H), 3045 (C–H), 1668 (C=O), 1605 (N–H). λ_{max} nm (MeOH): 222, 260, 402, and 469. Anal. Calcd for $RuC_{33}H_{27}N_7OCl_2$: C, 55.86; H, 3.84; N, 13.82. Found: C, 55.79; H, 3.77; N, 13.90.

Ru5. [Ru(phen)$_2$(na)]Cl.
Yield 45%. FTIR (KBr) cm^{-1}: 3068, 2922 (C–H), 1719, 1623 (C=O). λ_{max} nm (MeOH): 223, 265, 405, and 471. Anal. Calcd for $RuC_{36}H_{27}N_6O3Cl$: C, 59.38; H, 3.74; N, 11.54. Found: C, 59.25; H, 3.55; N, 11.60. ^{1}H NMR (DMSO-d6): δ = 9.44-9.43 (1H, d, *J* = 4.3 Hz), 9.28 (1H, s), 9.19 (1H, d, *J* = 4.4 Hz), 8.82 (2H, d, *J* = 7.45 Hz), 8.50–8.45 (2H, t, *J* = 17.4 Hz), 8.40–8.37 (3H, m), 8.35 (2H, d, *J* = 20.17 Hz), 8.23–8.19 (1H, dd, *J* = 8.18, 8.17 Hz), 8.11-8.06 (2H, m), 8.00-7.96 (2H, m), 7.57-7.50 (2H, m), 7.35 (1H, d, *J* = 8.3 Hz), 2.60 (3H, s, CH_3), 3.09 (2H, q, N-CH_2), 1.19 (3H, t, -CH_3).

Ru6. [Ru(bpy)$_2$(na)]Cl.
Yield 45%. FTIR (KBr) cm^{-1}: 3065, 2934 (C–H), 1716, 1621 (C=O). λ_{max} nm (MeOH): 225, 265, 402, and 480. Anal. Calcd for $RuC_{32}H_{27}N_6O_3Cl$: C, 56.51; H, 4.0; N, 12.36. Found: C, 56.44; H, 3.92; N, 12.48.

Ru7. [Ru(phen)$_2$(mitsz)]Cl$_2$.
Yield 41%. FTIR (KBr) cm1: 3450 (NH_2), 3225 (N-H), 2978 (C–H), 1677 (C=O), 1612 (N–H) and 1321 (C=S). λ_{max} nm (MeOH): 221, 263, 399, and 470. Anal. Calcd for $RuC_{34}H_{26}N_8SOCl_2$: C, 53.26; H, 3.42; N, 14.62. Found: C, 52.98; H, 3.39; N, 14.99. ^{1}H NMR (DMSO-d6): δ = 9.8 (1H, s), 9.68 (1H, d, *J* = 4.7 Hz), 9.23 (1H, d, *J* = 4.6 Hz), 8.81-8.74 (2H, m), 8.50-8.48 (2H, m), 8.39–8.37 (3H, m), 8.24–8.17 (3H, m), 8.13–8.11 (1H, m), 8.10–8.06 (1H, dd, *J* = 13.54, 8.19 Hz), 7.93 (2H, s, NH_2), 7.86-7.85 (1H, d, *J* = 4.38 Hz), 7.57-7.56 (1H, d, *J* = 4.41 Hz), 7.46-7.42 (1H, dd, *J* = 13.4, 8.1 Hz), 7.38-7.36 (1H, dd, *J* = 13.4, 8.0 Hz), 6.89-6.86 (1H, d, *J* = 8.1 Hz), 6.40-6.38 (1H, d, *J* = 7.76 Hz), 2.21 (3H, s, CH_3).

Ru8. [Ru(bpy)$_2$(mitsz)]Cl$_2$.
Yield 41%. FTIR (KBr) cm^{-1}: 3415 (NH_2), 3230 (N–H), 2975 (C–H), 1676 (C=O), 1610 (N–H) and 1319 (C=S). λ_{max} nm (MeOH): 220, 260, 400, and 475. Anal. Calcd for $RuC_{30}H_{26}N_8SOCl_2$: C, 50.14; H, 3.65; N, 15.59. Found: C, 50.09; H, 3.55; N, 15.81. ^{1}H NMR (DMSO-d6): δ = 9.79 (1H, s), 9.65-9.64 (1H, m), 9.20-9.19 (1H, m), 8.80 (1H, d, *J* = 7.74 Hz), 8.74-8.72 (1H, m), 8.47–8.45 (1H, m), 8.37–8.34 (3H, m), 8.22–8.15 (4H, m), 8.11–8.04 (2H, m), 7.90 (2H, m), 7.84-7.83 (1H, m), 7.43-7.40 (1H, m), 7.36-7.32 (1H, m), 6.86-6.84 (1H, m), 6.37-6.35 (1H, d, *J* = 7.7 Hz), 2.19 (3H, s, CH_3).

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 (20) 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). After 1 h virus adsorption period, residual virus was removed, and the cell cultures were 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.

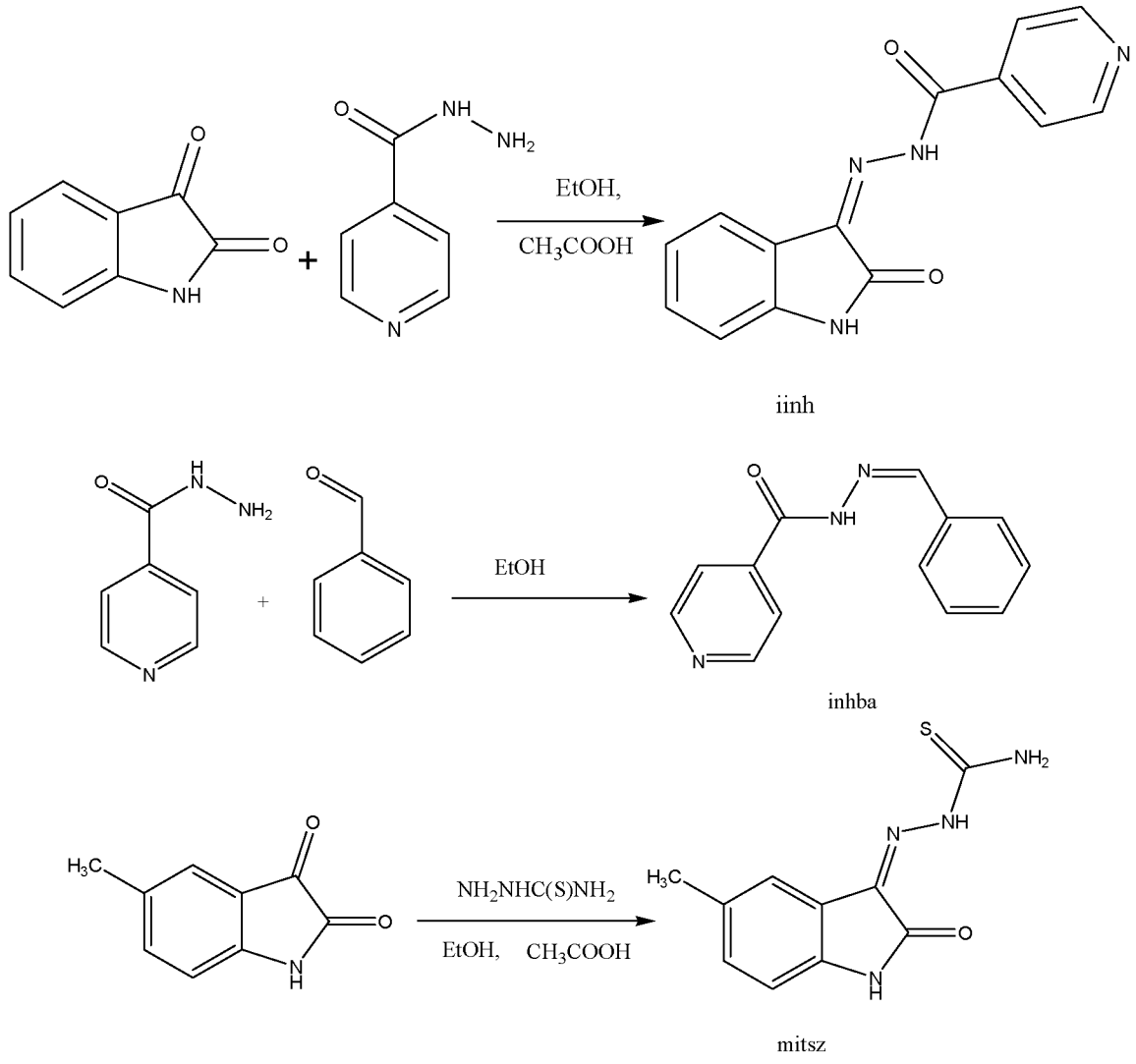
Cytostatic activity assays

The methodology for cytostatic activity assays in Molt 4/C8, CEM and L1210 assays has been published previously (21). Murine leukemia L1210 and human lymphocyte Molt4/C8 and CEM cells were seeded in 96-well microtiter plates at 50,000 (L1210) or 75,000 (CEM, Molt4/C8) cells per 200 µL-well in the presence of different concentrations of the test compounds. After 2 (L1210) or 3 (CEM, Molt4/C8) 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 inhibiting tumor cell proliferation by 50%.

RESULTS AND DISCUSSION

Chemistry

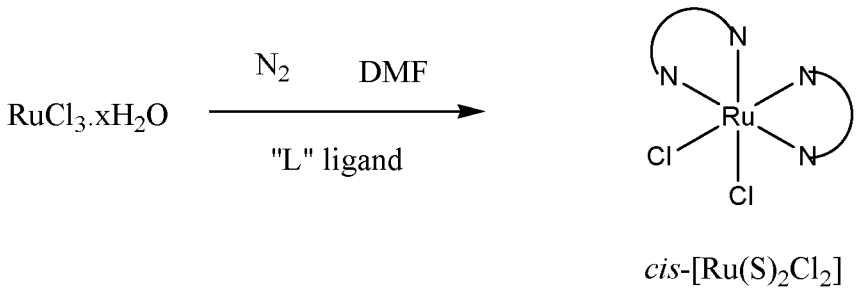
The ligand mitsz (5-methyl-isatin thiosemicarbazone) was prepared by reacting 5-methyl isatin with thiosemicarbazide in alcohol in the presence of acetic acid. Other ligands like inhba (N-benzyledeneisonicotinohydrazide) were prepared by reacting benzaldehydes with isonicotinohydrazide in alcohol at 1:1 molar ratio. The iinh [N-(2-oxindolin-3-ylidene) isonicotinohydrazide] ligand was prepared by reacting isatin with isonicotino- hydrazide in alcohol at 1:1 molar ratio in the presence of acetic acid (Scheme 1).



Scheme 1. Preparation of ligands (iitsz, mitsz and inhba)

All ligands were confirmed for their purity by their melting point, elemental analysis, and spectral studies. The details of the synthetic strategy adopted for the synthesis of these ruthenium homoleptic complexes were as follows. The starting material for the synthesis of the complexes was *cis*-bis(1,10-phenanthroline)dichlororuthenium(II)/*cis*-bis(2,2'-bipyridine) dichloro ruthenium(II).

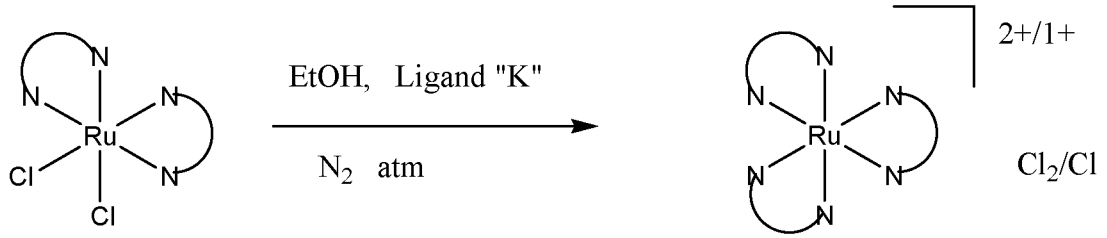
Ruthenium trichloride was refluxed in DMF in the presence of 1,10-phenanthroline/2,2'-bipyridine and in excess of the stoichiometric amount which afforded the final product *cis*-bis(1,10-phenanthroline)dichlororuthenium(II)/*cis*-bis(2,2'-bipyridine)dichlororuthenium(II) (Scheme 2). The third ligand was introduced in alcohol in the presence of nitrogen atmosphere (Scheme 3).



cis-[Ru(S)$_2$Cl$_2$]

Where S= 2,2'-bipyridine/ 1,10-phenathroline

Scheme 2. Preparation of *cis*-[Ru(S)$_2$Cl$_2$]

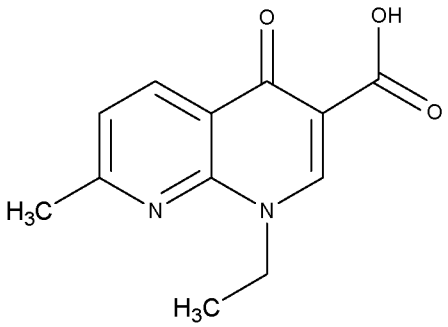


cis-[Ru(S)$_2$Cl$_2$]

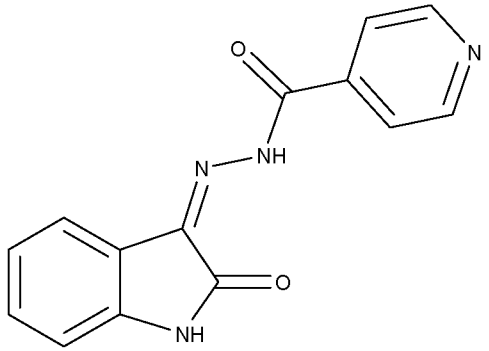
Ru1-Ru4 (2+)
Ru5,Ru6 (1+)
Ru7,Ru8 (2+)

Scheme 3. Preparation of tris chelates from *cis*-[Ru(S)$_2$Cl$_2$]

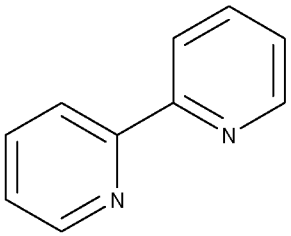
The ligands, especially iinh, inhba, na and mitsz, were capable of exhibiting bidentate behavior (Fig. 1).



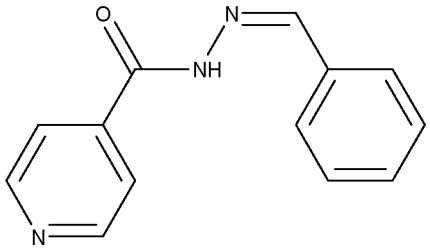
na: nalidixic acid



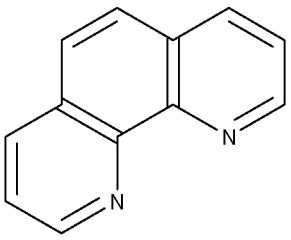
iinh: N-(2-oxindolin-3-ylidene)-isonicotinohydrazide



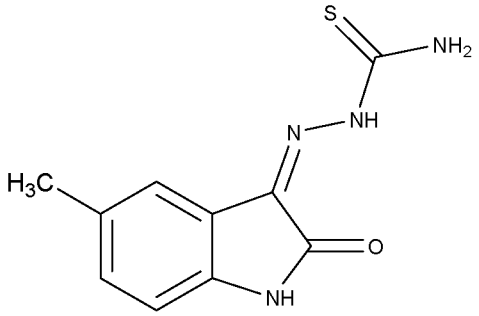
bpy: 2,2'-bipyridine



inhba: N-benzylideneisonicotinohydrazide



phen: 1,10-phenathroline



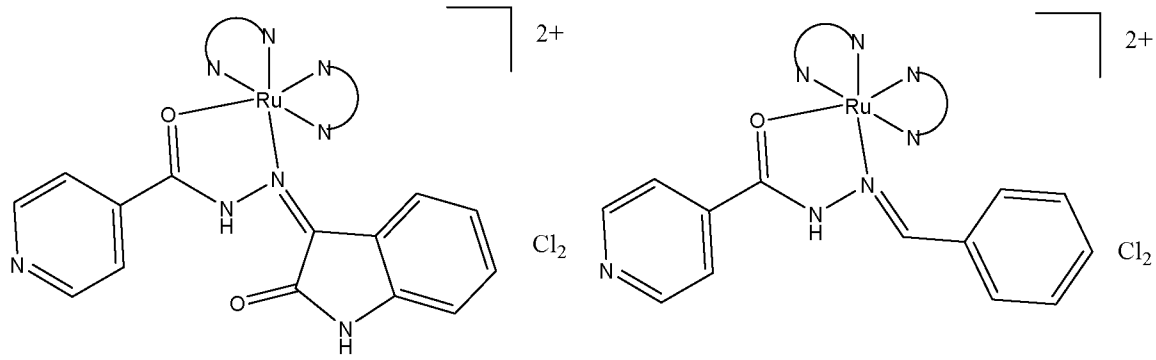
mitsz: 5-methylisatin thiosemicarbazone

Figure 1. Structures of bpy, phen, na, inhba and mitsz

There are very few cases in which the thiosemicarbazide acts as a monodentate ligand binding to the metal center through the sulfur atom. In the case of mitsz, the chelating mode was via a sulfur atom and imine nitrogen atom but not with one amide carbonyl oxygen. In other ligand (iinh), the chelating mode was *via* a carbonyl oxygen atom and an imine nitrogen by a coordination covalent bond. In the na ligand the covalent bond formed between the metal ion and the oxygen atom of the carboxylic group coordinate a covalent bond with the ring carbonyl oxygen. In case of the inhba ligand the chelating mode was via the carbonyl oxygen and the imine nitrogen but not with the isatin carbonyl oxygen. The infrared spectra of all the ligands and their ruthenium (II) complexes were recorded in KBr powder by diffuse reflectance

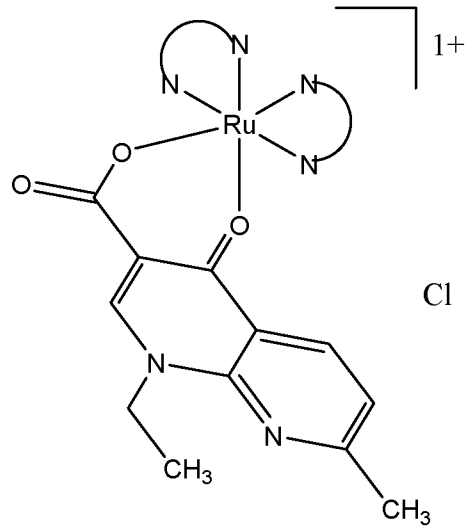
technique and are reported in their respective titles by tentative assignments. The mitsz ligand showed vibrational frequency from 3450 to 3150 cm^{-1} which was assigned for NH_2 and N–H stretching and at 1675 cm^{-1} for amide carbonyl group and at 1363 cm^{-1} for C=S stretching. The iinh ligand showed vibrational frequency, respectively from 3397 to 3371 cm^{-1} for N-H stretching and from 1690 and 1643 cm^{-1} for C=O stretching. The inhba ligand showed vibrational frequency at 3365 cm^{-1} for NH stretching and at 1698 cm^{-1} for C=O stretching. The na ligand showed vibrational frequency from 3500 to 3130 cm^{-1} for OH (bonded) stretching and from 2934 to 2922 cm^{-1} for C-H aliphatic stretching and 1720, 1621 cm^{-1} for C=O stretching.

A comparison of IR spectra of ligand mitsz with ruthenium complexes (Ru7, Ru8) indicates this was coordinated to the metal center by sulfur and imine nitrogen but not with amide carbonyl oxygen and not with terminal amine group, which was confirmed by the IR spectra, which indicates no change in vibrational frequency of amide carbonyl group at 1677 cm^{-1} and at 3450 cm^{-1} for amine group. In the IR spectra of na and its complexes Ru5 and Ru6, the bond formation took place between oxygen of hydroxyl group and ring carbonyl oxygen, which was confirmed by the absence of the O–H vibration peak. In the IR spectra of inhba and its complexes Ru3 and Ru4, the bond formation took place between oxygen of carbonyl group and imine nitrogen, which was confirmed by the change in vibrational frequency for C=O at 1673-1668 cm^{-1}. In the IR spectra of iinh and its complexes Ru1 and Ru2, the bond formation took place between the carbonyl oxygen of inh and the imine nitrogen of isatin, which was confirmed by the change in vibrational frequency at 1618 cm^{-1} for C=O. Coordination of ligands (K = iinh, inhba, na, mitsz) to ruthenium results in compounds such as $[Ru(S)_2(K)]^{2+}Cl_2$ (Ru1–Ru8). All these complexes do not possess any C_2 axes of symmetry. Such a loss of C_2 axis of symmetry was seen for $[Ru(L)_2(R)]$ (where L = 2,2'-bipyridine/1,10-phenanthroline, whereas K = acetazolamide, 7-iodo-8-hydroxy-quinoline, 4-substituted thiopicolinanil- ide, etc.) (15). All compounds had well-resolved resonance peaks, which correspond to four different aromatic ring protons of the two 2,2'-bipyridine/1,10-phenanthroline ligands and the third ligand. These complexes showed broad and intense visible bands between 350 and 500 nm due to metal to ligand charge transfer transition (MLCT). In the UV region the bands at 290 and 310 nm were assigned to 2,2'-bipyridine/1,10-phenanthroline ligand $\pi-\pi^*$ charge transfer transitions. The same transition was found in free 2,2'-bipyridine/1,10-phenanthroline at 280 nm, so that coordination of the ligand resulted in a red shift in the transition energy. There were also two shoulders at 390 and 500 nm, which were, tentatively, attributed to metal to ligand charge transfer transitions involving 2,2'-bipyridine, 1,10-phenanthroline, and the third ligand. Thus, based on the above observations, it is tentatively suggested that Ru(II) complexes showed an octahedral geometry (Fig. 2).

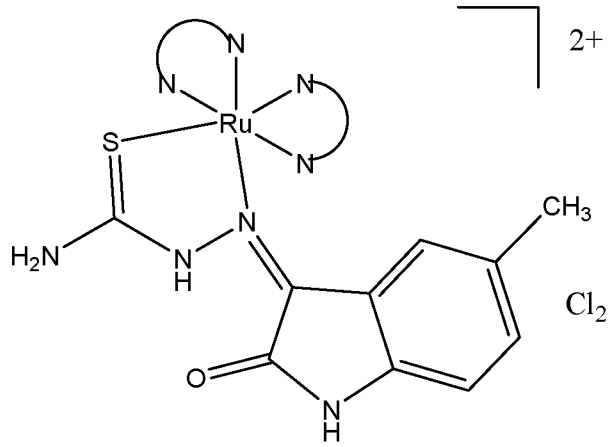


Ru1:N-N=1,10-phenanthroline
Ru2:N-N=2,2'-bipyridine

Ru3:N-N=1,10-phenanthroline
Ru4:N-N=2,2'-biprydine



Ru5:N-N=1,10-phenanthroline
Ru6:N-N=2,2'-bipyridine



Ru7:N-N=1,10-phenanthroline
Ru8:N-N=2,2'-bipyridine

Figure 2. Proposed structures for the ruthenium complexes [Ru1-Ru8]

Biological activity

The in vitro cytotoxic activity was evaluated for ruthenium complexes against human Molt 4/C8 and CEM T-lymphocytes as well murine leukemia L1210 cells. Cells were seeded in 200 µl-microtiter plate wells in RPMI-1640 culture medium (supplemented with fetal calf serum (10%), 2 mM L-glutamine and 0.075% $NaHCO_3$) in the absence or presence of serial concentrations (200, 40, 8, 1.6 and 0.32 µg/mL) of the test compounds. The cell number was counted after 2 (L1210) or 3 (CEM, Molt4/C8) days using a Coulter counter. The results are presented in Table 1.

Table 1. Inhibitory effects of complexes on the proliferation of murine leukemia (L1210) and human T-lymphocyte cells (Molt4/C8, CEM).

Complexes	IC_{50}* (μg/mL)		
	L1210	**Molt4/C8**	**CEM**
Ru1	92 ± 1	117 ± 27	89 ± 28
Ru2	100 ± 5	132 ± 35	78 ± 49
Ru3	15 ± 4	3.7 ± 0.4	0.84 ± 1.04
Ru4	35 ± 20	22 ± 6	5.9 ± 4.7
Ru5	91 ± 5	120 ± 13	8.2 ± 3.6
Ru6	98 ± 3	158 ± 47	53 ± 29
Ru7	16 ± 7	23 ± 13	18 ± 0
Ru8	≥ 200	> 200	119 ± 67
Melphalan	2.1 ± 0.02	3.2 ± 0.6	2.5 ± 0.2

*50% inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50 %.

The cytostatic data revealed that most ruthenium complexes have antiproliferative potencies that widely vary depending on the nature of the compound (IC_{50}'s ranging from 0.84–119 μg/mL against CEM, 3.7– >200 μg/mL against Molt 4/C8, and 16– ≥200 μg/mL against L1210). Of the tested ruthenium complexes, Ru3 showed the most pronounced antiproliferative activity against all three cell lines tested in the range of 0.84 to 15 μg/mL on comparison to standard melphalan 2.1 to 3.2 μg/mL. There fore Ru3 is three times more potent (0.84 μg/mL) and almost equipotent (3.7 μg/mL) against Molt and CEM cell lines respectively than the standard melphalan (2.5 μg/mL and 3.2 μg/mL). Where as Ru5 was cytostatic against CEM cells at 8.2 μg/mL than the other tumor cells 91-120 μg/mL. Ru7 showed cytostatic activity for all three tested cell lines in the range of 16- 23 μg/mL.

The compounds have also been evaluated for their inhibitory activity against a wide variety of DNA and RNA viruses (see experimental procedures), but were found inactive at subtoxic (20-100 μg/mL) concentrations.

CONCLUSION

In conclusion, eight ruthenium (Ru1–Ru8) complexes, bearing 2,2'-bipyridine and 1,10-phenanthroline with mitsz, inhba, na and iinh were synthesized. The coordination for Ru1, Ru2, Ru3 and Ru4 compounds was *via* the C=O and imine nitrogen, but not with the amide carbonyl functional group of the iinh and inhba ligand, respectively. For ruthenium compounds Ru5–Ru6 the coordination involved was with C=O. In case of ruthenium compounds (Ru7 and Ru8), the coordination involved was *via* C=S and the imine nitrogen, but not with the amide carbonyl functional group of the mitsz ligand. From the results presented in Table 1, it is clear that several ruthenium compounds exhibited a marked inhibitory effect on the proliferation of tumor cells. Among the tested compounds, Ru3 emerged as most potent cytotoxic compound against CEM cell lines at 0.84 μg/mL and which is three times more potent than the standard melphalan (2.5 μg/mL). However, the mechanism of cytostatic/toxic action is currently unclear and should be subject of further investigations.

ACKNOWLEDGMENT

Authors are thankful to Dr. B. G. Desai, Principal, KLE University's College of Pharmacy, Bangalore for providing necessary facilities. The authors also thank Mrs. Lizette van Berckelaer, Leentje Persoons, Frieda De Meyer and Vicky Broeckx for excellent technical assistance. Appreciation is extended to the Belgian Geconcerteerde Onderzoeksacties (GOA 05/19) which supported the cytostatic activity assays.

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Received: 27.09.2010
Accepted: 03.11.2010