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Ru(II) COMPLEXES AS CYTOTOXIC AGENTS: SYNTHESIS AND STRUCTURAL CHARACTERIZATION

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ABSTRACT

We report on the synthesis and structural characterization of ruthenium complexes (Ru-1 to Ru-4) of the type $[Ru(S)_2(K)]^{2+}$, (Where S=1,10-phenanthroline/2,2'-bipyridyl and K=dmhc, dmih where dmhc=2-(diphenylmethylene)hydrazincarbothioamide,dmih=N'(diphenylmethylene) isonicotinohydrazide are described. These ligands form bidentate octahedral ruthenium complexes. The *in vitro* cytotoxic of the complexes measurement against the human cancer T-lymphocyte cell lines. In vitro evaluation of these title complexes revealed cytotoxicity from 1.2 to 5.4 µg/mL against CEM, 0.98 to 3.4 µg/mL against L1210, 1.4 to 4.8 µg/mL against Molt 4/C_8, 0.84 to 2.6 µg/mL against HL 60 and 1.6 to 3.4 µg/mL against BEL7402.

KEYWORDS: Characterization, Cytotoxicity, Ruthenium complexes.

INTRODUCTION

Metal complexes provide a highly versatile platform for drug design. Researchers are searching for the antitumor active metal complexes; several platinum complexes have been reported to be promising as antitumor drugs. Development of anticancer drugs with fewer or no side effects is important for the treatment of cancer. Research in this area is expanding rapidly and some promising leads have emerged. Metal and metal ions are showing a good effect on cellular process. These metals influence not only natural processes such as cell division and gene expression but also some other process, such as toxicity, carcinogenicity and antitumor chemistry. [1] Based on the above investigations a special aspect of metal

biochemistry, namely the kinetics and reactivity of metal coordination complexes in living systems, with a focus on heavy metals like Pt & Ru and their antitumor action. [2]

Since the serendipitous discovery of the biological activity of cisplatin in 1965 [3] and its subsequent clinical use for the treatment of various solid tumours including colorectal, genitourinary, and non-small cell lung cancers.[4] Medicinal inorganic chemistry has become a subject of intensive studies and continues to attract much attention in the drug discovery field. [5,6] However, the clinical use of cisplatin is largely restricted by dose-limiting side effects such as neuro-, hepato- and nephrotoxicity, and inherent or acquired resistance. During the past three decades, therefore, continuous effort has been devoted to the development of new platinum drugs and other metal-based, in particular ruthenium based anticancer drugs to circumvent these limitations. [7, 8, 9, 10]

A series of ruthenium complexes having the general formula $[Ru(S)_2(K)]$, where S=2,2′-bipyridine/ 1,10-phenanthroline and K= hfc, itsz, Meo-btsz, 4-Cl-btsz *etc*., reported. [11] Complex $[Ru(Phen)_2(p\text{-}MOPIP)]^{2+}$ can effectively inhibit the proliferation of Hep G-2 cell line with low IC_{50} value (7.2 to 1.3µM). [12] $[Ru(Phen)_2 (DBHIP)]^{2+}$ can effectively induce apoptosis of BEL-7402 cell lines [13]. In recent year, several ruthenium based complexes have been investigated such as chiral ruthenium complex [(1s, 2s)-DEPN]–$RuCl_2(PPh_3)_2$[14], new chiral – bridged diamine / diphosphine Ru(II) complexes [15] Chlorido-(p-cymene)-[(1,3-dimethyl-4-(1-naphthoyl)-pyrazolon-5-ato]ruthenium(II)[16], (1,4,7,10,13–penta thio cyclo pentadecane) chloro ruthenium (II) hexa fluoro phosphate [17], $[Ru(phen)_2(mitatp)]^{2+}$. [18] $[Ru(bpy)Br_2(acac)](PF_6)$ [19]., antiviral activity of ruthenium(II) arene complexes [20]. Recently we reported Ruthenium (II) complexes as anticancer activity. [21, 22, 23, 24]

In this report, we evaluated the complexes type $[Ru(S)_2(K)]2+$, (Where S=1, 10-phenanthroline/2, 2'-bipyridyl and K= dmhc and dmih where dmhc=2-(diphenylmethylene) hydrazincarbothioamide, dmih=N'-(diphenylmethylene) isonicotinohydrazide for cytotoxic activities. The *in vitro* cytotoxic and activitiy of the complexes measurement against the human cancer T-lymphocyte cell lines.

Experimental

Materials for Synthesis: All reagents and solvents were purchased from Sigma-Aldrich and used as received. The $RuCl_3.3H_2O$ was purchased from Sigma-Aldrich. The ruthenium compounds Ru-1 to Ru-4 were prepared using the synthetic strategy describes **Schemes 1-3**.

The synthesis began by preparation of thiosemicabzone, isonicotinyl hydrazones and ligands. The ligands were prepared according to the published procedures. [25] The next step was performed by commercially available ruthenium trichloride with 1, 10–phenanthrolene/2, 2-bipyridyl. The final ruthenium complexes were synthesized by treating $[Ru(phen)_2Cl_2]$ with dmhc, dmih ligands to offered the corresponded complex.

Synthesis

General procedure for preparing $[Ru(S)_2(K)Cl_2]$ (where S=2,2-bipyridine/ 1,10-phenanthroline; K= dmhc and dmih.

To the black microcrystalline *cis*-bis (A) dichlororuthenium(II) {*cis*-$Ru(S)_2Cl_2$} (2mmol) excess of ligand K (2.5 mmol) was added and refluxed in anhydrous ethanol under nitrogen. The initial colored solution slowly changed to brownish orange at the end of the reaction, which was verified by TLC on silica plates. Then excess ethanol was distilled off and silicagel (60-120 mesh) added to this solution. The final complex was purified by column chromatography by using silica gel as stationary phase and chloroform-methanol as mobile phase.

Characterization of Synthesized Ruthenium (II) complexes

Ru-1: $[Ru(phen)_2(dmhc)]Cl_2$.

51%, black crystals, IR (KBr) cm^{-1}: 3456-3322 (NH_2 & N-H), 3018 (C-H) 2965 (C-H), 1324 (C=S). Calcd. For $C_{38}H_{29}N_7RuS$: C, 63.67; H, 4.08; N, 13.68. Found C, 63.12; H, 4.04; N, 13.62%. ^{1}H-NMR (DMSO-d_6): δ ppm: 9.28 (s, 1H), 8.96 (s, 1H), 8.88 (s, 1H), 8.72 (d, 2H), 8.54 (d, J = 4.9 Hz, 2H), 8.44 – 8.32(d, J = 5.0 Hz, 2H), 8.22 (s, 1H), 8.08 (s, 1H), 7.96 (s, 1H), 7.84 (m, 4H), 7.70- 7.54 (m, 4H) 7.56 (dd, 2H) 7.38 (s, 1H), 7.21(m, 3H), 6.96 (d, 2H),6.54 (s, 1H), FAB-MS (mNBA): 716 $[Ru(phen)_2(dmhc)]^{2+}$; 461 $[Ru(phen)_2]$; 255 [dmhc].

Ru-2: $[Ru(bpy)_2(dmhc)]Cl_2$.

49%, black crystals, IR (KBr) cm^{-1}: 3430-3298 (NH_2 & N-H), 3098 (C-H) 2954 (C-H), 1328 (C=S). Calcd. For $C_{38}H_{29}N_7RuS$: C, 61.06; H, 4.37; N, 14.66 Found C, 60.92; H, 4.32; N, 14.62%. ^{1}H-NMR (DMSO-d_6): δ ppm: 9.22 (s, 1H), 9.14 (s, 1H), 9.06 (s, 1H), 8.98 (s,1H), 8.84 (s, 1H), 8.52 (d, 2H), 8.42 (d, 2H), 8.34 – 8.26(d, J = 5.0 Hz, 2H), 8.14-8.02 (dd, 2H), 8.0 (s, 1H, NH), 7.88 (t, 3H), 7. 74-7.68(m, 3H), 7.62 (d, J = 14.2 Hz, 2H), 7.52- 7.46 (m,

3H) 7.36 (dd, 2H) 7.16 (d, 2H). FAB-MS (mNBA): 668 $[Ru(bpy)_2 (dmhc)]^{2+}$; 413 $[Ru(bpy)_2]$; 255 [dmhc].

Ru-3: $[Ru(phen)_2(dmih)]Cl_2$.

46%, black crystals, IR (KBr) cm^{-1}: 3288 (N-H) 2965 (C-H), 1680 (C==O). Calcd. For $C_{43}H_{31}N_7RuO$: C, 67.70; H, 4.10; N, 12.85. Found C, 67.48; H, 4.06; N, 12.76%. ^1H NMR (DMSO-d_6): δ ppm: 9.32 (s, 1H), 9.18 (s, 1H), 8.98 (s, 1H), 8.84 (s, 1H), 8.76 (s, J = 4.9 Hz, 1H), 8.68 (d, J = 8.4 Hz, 2H), 8.46 (d, 2H), 8.68 (d, 2H), 7.94 (d, J = 5.0 Hz, 2H), 7.86 (m, 3H),7.75-7.64 (m, 4H) 7.52 (d, 2H), 7.36 (d, 2H), 7.26 (d, J = 14.6 Hz, 2H), 7.18 (s, 1H), 6.92 (s, 1H), 6.81-6.78 (d, 2H) 6.75 (s, 1H). FAB-MS (mNBA): 762 $[Ru(phen)_2 (dmih)]^{2+}$; 413 $[Ru(phen)_2]$; 301[dmih].

Ru-4: $[Ru(bpy)_2(dmih)]Cl_2$.

52%, black crystals, IR (KBr) cm^{-1}: 3302 (N-H) 3024 (C-H), 1682 (C==O). Calcd. For $C_{39}H_{31}N_7Ru_1O$: C, 65.53; H, 4.37; N, 13.72. Found C, 65.38; H, 4.34; N, 13.66 %. ^1H NMR (DMSO-d_6): δ ppm: 9.44 (s, 1H), 9.26 (s, 1H), 8.95 (s, 1H), 8.84 (s, 1H), 8.78 (s, J = 4.9 Hz, 1H), 8.65-8.43 (d, J = 8.4 Hz, 2H), 8.42-8.26 (d, 2H), 8.22 (d, 3H), 8. 01-7.98 (m, 3H), 7.84 (d, J = 4.9 Hz, 2H), 7.80-7.62 (m, 4H), 7.54-7.52 (d, 2H), 7.48 (s, 1H), 7.24 (d, J = 14.8 Hz, 2H), 7.12 (s, 1H), 6.96 (s, 1H), 6.88-6.72 (d, 2H) 6.68 (s, 1H). FAB-MS (mNBA): 714 $[Ru(bpy)_2 (dmih)]^{2+}$; 413 $[Ru(bpy)_2]$; 301 [dmih].

3. RESULTS AND DISCUSSION

The compounds of the newly synthesized ruthenium compounds were confirmed by UV-Vis, FT-IR, ^1H-NMR, Mass spectroscopy and Elemental analysis. In the UV-Vis spectra all the ruthenium compounds showed broad and intense visible bands between 330 and 530 nm due to metal to ligand charge transfer transition (MLCT). In the UV region the bands at 285 and 320 nm were assigned to 1, 10-phenanthroline ligand π–π^* charge transfer transitions. The IR spectras contained the absorption bands revealing the existence of the NH_2, NH, C=S and C=O gps. The ^1H-NMR spectra of the complex, $[Ru(phen)_2(dmhc]Cl_2$ shown 29 resonance peaks (9.22-7.16). The mass spectra of the Complex Ru-1 (see the supporting information) gave the anticipated molecular ion peak and main fragmentation peaks, which were in accordance with the title complexes. The *in vitro* antineoplastic activities of the synthesized complexes against the human cancer T-lymphocyte cell lines Molt 4/C_8 and CEM and the murine tumor leukemia cell lines L1210, human oral epidermoid carcinoma KB cells, human

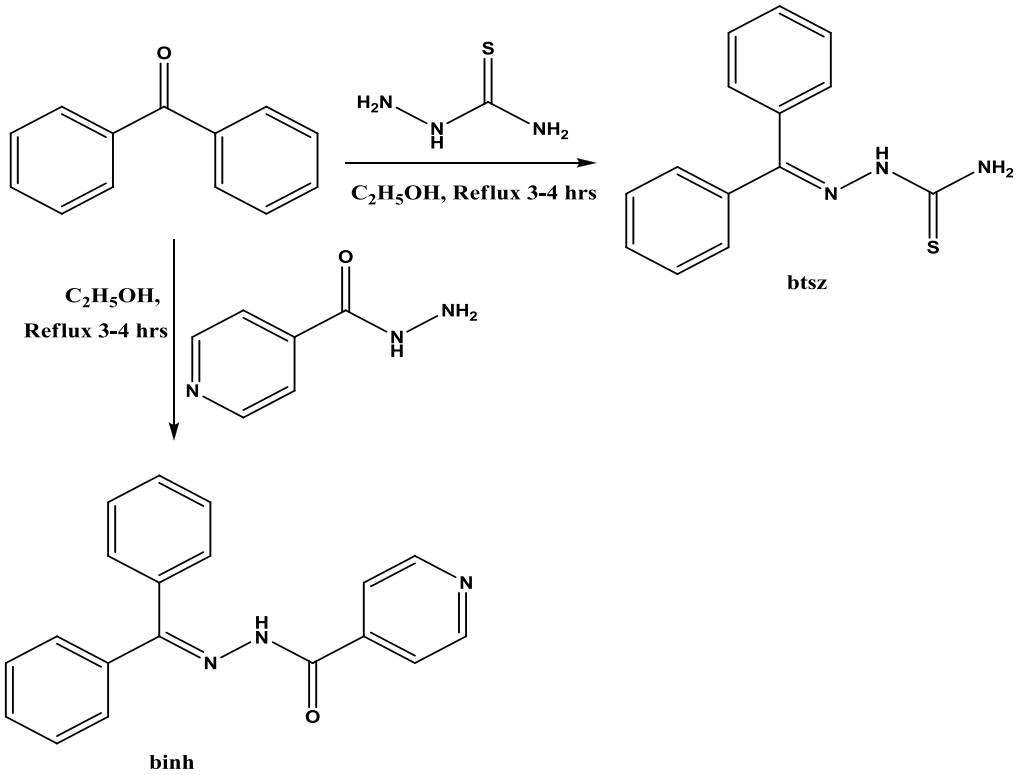
promyelocytic leukemia cells (HL60) and Bel-7402 liver cancer cells were evaluated by the standard MTT assay. [26-28] As described in Table 1, complexes Ru-1, Ru-2, Ru-3 and Ru-4 exhibit very potent cytotoxic activity against all the cell lines, especially **Ru-1** shown very potent antitumor activity like cisplatin and shows good selectivity. On comparison to ruthenium compounds, the ligands displayed the cytotoxicity at higher concentration. Thus, the ruthenium compounds proved inhibitory to tumor growth at submicromolar concentration.

Table 1

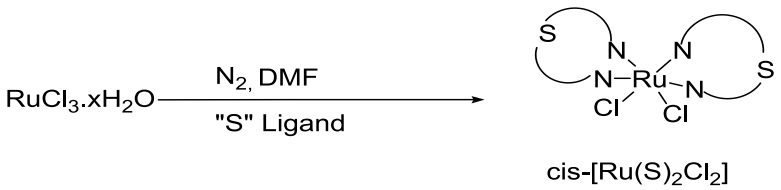
Cytotoxic studies of ruthenium complexes.

Comp. code	IC_{50}[a] (µmol/L)				
	CEM	L1210	Molt 4/C_8	HL60	BEL7402
bptsz	210 ± 18	198 ± 14	168 ± 12	202 ± 18	158 ± 16
bpinh	248 ± 34	240 ± 06	228 ± 06	214 ± 25	198 ± 08
Ru-1	1.2 ± 0.4	0.98 ± 0.6	1.4 ± 04	0.84 ± 2.6	1.6 ± 12
Ru-2	2.3 ± 0.8	1.8 ± 12	2.5 ± 08	2.0 ± 12	3.4 ± 08
Ru-3	4.2 ± 04	3.4 ± 1.2	4.6 ± 02	2.6 ± 08	2.8 ± 06
Ru-4	5.4 ± 02	2.8 ± 0.6	4.8 ± 14	2.2 ± 0.4	3.2 ± 0.8
Cisplatin	0.51 ± 0.1	1.2 ± 0.02	0.87 ± 0.06	0.98 ± 0.02	0.78 ± 0.04

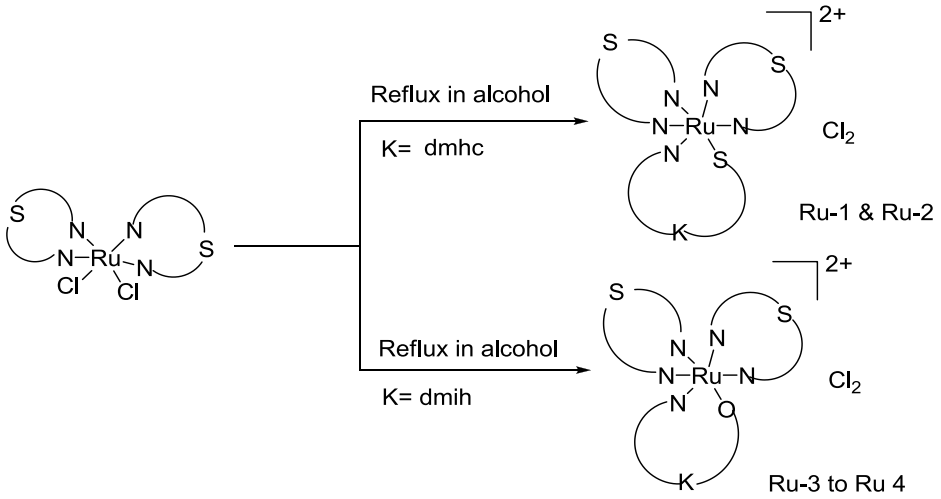
[a]50% inhibitory concentration, required to inhibit tumor cell proliferation by 50%



Scheme 1. Synthesis of dmhc, dmih ligands.



Scheme 2. Synthesis of *cis*-[Ru(S)$_2$ Cl$_2$] Where S = 1, 10-Phenanthroline/2-2'-bipyridyl.



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

CONCLUSION

In summary, we described the synthesis of novel ruthenium(II) Complexes bearing dmhc and dmih derivatives. These ruthenium compounds possess excellent *in vitro* cytotoxic activities.. With the increasing competition and reducing development time companies are forced to adopt strong techniques for many block buster molecules and new chemical entities (NCEs). This invention provides a novel class of ruthenium compounds that exhibited anticancer activity.

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