International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 7 (1): 96-105, 2011
ISSN 1811-7775 / DOI: 10.3923/ijp.2011.96.105

Investigation of Genotoxic Effects of Some Ruthenium Complexes According to Cis-platinum

[1]F.S. Alanyali, [1]E. Ergin, [1]O. Artagan and [2]K. Benkli
[1]Department of Biology, Faculty of Science,
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy,
Anadolu University, 26470, Eskişehir, Turkey

Abstract: The aim of our study was, to synthesize and evaluate the mutagenicity of Ruthenium complexes, on *Salmonella* strains of TA100 and TA98 by Ames Test. $[Ru(L)_2(B)Pt]$ Cl_2 complexes were synthesized using 1, 10-phenanthroline-5,6-dione (dp) (L1), imidazo [4,5-f] [1,10] phenanthroline (ip) (L2) and 2-phenylimidazo [4,5-f] [1, 10] phenanthroline (pip) (L3) as ligands and bis-1,4-di [([1, 10] phenantrolin-5-il) amino]-2-buten (B) as a bridge molecule. The characterization of the intermediate and final compounds arising from this work will be carried out by means of a variety of spectroscopic methods, which include ^{1}H NMR, IR, MS and elemental analysis. The mutagenicity of $[Ru (L)_2 (B) Pt] Cl_2$ complexes, was investigated by the Ames Salmonella assay with strains TA 98 carrying a frameshift mutation and TA 100 base-pair substitution mutation were used in plate incorporation method in the absence of metabolic activation. RuL_2BPt complex on TA98 *Salmonella* strain was found mutagenically effective at the dose of 100 μg/plt. RuL_3BPt complex gave mutagenic response on TA98 strain at doses of 25, 50, 100 μg/plt, maximum mutagenic response was obtained at 25 μg/plt dose. There was no significant increase of revertants due to the treatment with the doses of 5, 10, 25, 50 μg/plt cis-platin on TA98. 100 μg/plt dose showed mutagenic effect on TA98. On TA100 strain dose related increase in the number of revertant colonies was observed but the number of revertants were not double the number of spontaneous colonies up to 100 μg/plt dose. Only 100 μg/plt dose showed mutagenic effect. The test compounds were directly effective, especially in the TA 98 Strain of *Salmonella*. Ruthenium derivatives was not mutagenic on the *Salmonella* strain of TA100.

Key words: Ru (III) complexes, mutagenicity, ames test, *Salmonella typhimurium*, cisplatin

INTRODUCTION

Most drugs used nowadays have considerable side effects, such as development of resistance of cancer cells to the cytostatic agents. Therefore new strategies are developed in the synthesis of new cytotoxic and therapeutic agents and in some cases the complexes by metal-ligand bonding derivatives were synthesized. Especially cisplatin is a highly effective anticancer drug. The efficiency of cisplatin is based on its ability to bind to genomic DNA. The two chlorine atoms of the cisplatin molecules are replaced by N atoms of adjacent purine bases and most of the well-known platinum anticancer complexes have at least one N-H group, which is responsible for important hydrogen-bond donor properties (Mock *et al*., 2001; Reedijk, 2003). Ligands possessing hydroxyimino groups together with other powerful donor groups can be very efficient chelating agents which are be able to facilitate the stabilization of high oxidation states of 3d-metals.

Recently there has been increased attention focused on the binding properties of metal complexes, particularly polypyridyl complexes of ruthenium, with biomolecules like DNA, RNA or polynucleotides (Barton and Lolis, 1985; Ortmans *et al*., 1998; Pyle and Barton, 1990; Ambroise and Maiya, 2000; Chao *et al*., 2002; Dandliker *et al*., 1997; Patel *et al*., 2002; Zhen *et al*., 2000; Vaidyanathan and Nair, 2002). Cationic metal complexes possessing planar aromatic ligands may bind to DNA by intercalation, which involves stacking of the planar ligand in between adjacent base pairs of the DNA duplex (Pinedo and Schornagel, 1996; Loehrer and Einhorn, 1984; Maheswari and Palaniandavar, 2004; Qu and Chaires, 2001; Kelly *et al*., 1999). Such metallointercalators tend to be strongly mutagenic and some have shown promising chemotherapeutic activity, which correlates well with DNA binding affinity (Komeda *et al*., 2000; Novakova *et al*., 1995). They have been useful in probing DNA structure and function and the intercalation process itself, because the ligands of metal may be varied in an easily controlled manner to facilitate the individual application. Certain metallointercalators cleave DNA owing to the redox activity of the metal center.

Corresponding Author: F.S. Alanyali, Department of Biology, Faculty of Science, Anadolu University, 26470, Eskisehir, Turkey Fax: +90-222-3204910

Octahedral substitution-inert and redox-active ruthenium (II) complexes, incorporating aromatic planar bidentate ligands have many unique and convenient features such as strong visible absorbance in the visible range (Sitlani *et al*., 1992; Farrell, 2002; Arounagiri *et al*., 2000; Garoufis *et al*., 2003; Jakson and Barton, 2000; Tuite, 1998; Stemp *et al*., 2000; Collins *et al*., 1999; Holmlin *et al*., 1999; Watt *et al*., 1996). Many amine complexes of Ru (II) tend to selectively bind to imine sites in biomolecules, because of their nitrogen lone pairs available for metal ion coordination. Consequently, ruthenium complexes often selectively coordinate histidyl imidazole itrogens on proteins and the N7 site on the imidazole ring of purine nucleotides (Gray and Winkler, 1996; Messori *et al*., 2000a; Messori *et al*., 2000b; Rodriguez-Bailey *et al*., 1997; Clarke, 2002; Deng *et al*., 2005). In comparison with other complexes, the octahedral polypyridyl ruthenium complexes are much more suitable, because they are coordinatively saturated and inert to substitution. These complexes bind to DNA mainly through three types of weak interactions, these are electrostatic-dominated binding, involving interactions between the cationic metal complex and the negatively charged phosphates of the DNA, ligand π-stacking interactions, that are characterized by intercalation of an extended electron-deficient planar aromatic ring system (two or three six-membered rings), adjacent between the base pairs, through the major or the minor groove of the nucleic acid helix; and groove binding, in which the metal complex binds in the DNA grooves, associated by hydrogen bonds and/or Van der Waals interactions along the groove of the duplex. In contrast to the intercalation mode, the groove binding does not significantly perturb the DNA structure (Zimmer and Wahnert, 1986; Devran, 1986).

The Ames Test System is a short-term bacterial reverse mutation assay widely used to detect the mutagenicity of chemicals. In this test histidine dependent *Salmonella* strains carrying different mutations in various genes in the histidine operon (Maron and Ames, 1983; Waters *et al*., 1990; Mortelmans and Zeiger, 2000). In the last several years ruthenium derivatives have been proposed as potential antitumor agents because of their cytotoxic and mutagenic effects. These complexes bind to DNA strands and inhibit DNA replication and transcription (Grguric-Sipka *et al*., 2003; Clarke, 2003; Benkli *et al*., 2009; Wang, 1985; Chen *et al*., 1993).

MATERIALS AND METHODS

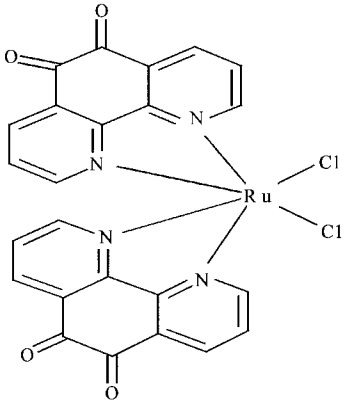
Place and duration of the study: This research was carried out between April 2007-November 2009, at the Department of Biology, Faculty of Science, and Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University.

Chemistry: $RuCl_3.(H_2O)n$, LiCl and 1,10-phenanthroline-5,6-dione were purchased from Aldrich organics. All other reagents were used as purchased from commercial suppliers without further purification. The compounds were checked for purity by TLC on silica gel 60 F_{254} (Merck). Melting points were determined by using a Gallenkamp MPD 350. BM 2.5 digital melting point apparatus and were uncorrected. Spectroscopic data were recorded on the following instruments: Elemental analyses were performed on a CHNS-O Carlo Erba EA 1108 elemental analyzer IR with a Shimadzu 470 IR spectrophotometer Anadolu University, Turkey; ^{1}H NMR spectra (δ (ppm) Hz) were run on a Varian (300 MHz) spectrometer in CDCl3 or d6-DMSO; Fast atom bombardment (FAB) was recorded with a Finnigan Mat 95 mass spectrometer with meta-nitrobenzylalcohol as matrix. The compounds were checked for purity by TLC on silica gel 60 F254 (Merck).

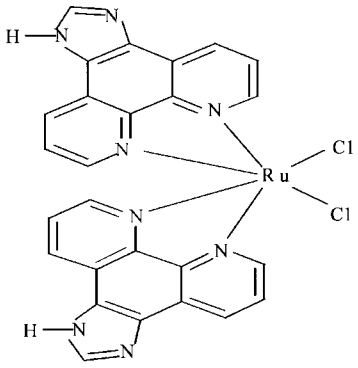
Synthesis of the ligands and the bridge: The compound 1,10-phenanthroline-5,6-dione (dionphen) (L1) were bought. The ligands imidazo [4, 5-f] [1,10] phenanthroline (ip) (L2) and 2-phenylimidazo [4, 5-f] [1,10] phenanthroline (pip) (L3) were synthesized according to published procedures already (Paw and Eisenberg, 1997; Yamada *et al*., 1992; Wu *et al*., 1997; Xiong *et al*., 1999). A mixture of 1,10-phenanthroline-5,6-dione (1 mmol), an appropriate aldehyde derivative (1.4 mmol), glacial acetic acid (8 mL^{-1}) and ammonium acetate (1.6 g) was refluxed for 1 h; after cooling, this mixture was diluted with water and neutralized with conc. aqueous ammonia, immediately resulting in a yellow or light-yellow precipitate, which was washed with water, acetone and diethyl ether respectively and then dried in a desiccator. The pure products were obtained by recrystallization. The bridge compound bis-1,4-di [([1,10] phenantrolin-5-il) amino]-2-buten (B) was obtained using published methods (March, 1985; Alp *et al*., 2009). Purity of the compounds was checked controlled by thin layer chromatography.

Synthesis of RuL_2Cl_2 complexes: A mixture of $RuCl_3.nH_2O$ (1 mmol), the appropriate phenanthroline derivative (2 mmol), LiCl (7 mmol) and DMF (8 mL^{-1}) was refluxed under argon for 8 h during which the color of the solution changed to dark. The reaction mixture was cooled, acetone was added and the mixture was kept at 0°C for 24 h. The precipitate was filtered, washed with water and diethylether and dried in vacuo (Jiang *et al*., 2001; Wu *et al*., 1997a; Paw and Eisenberg,

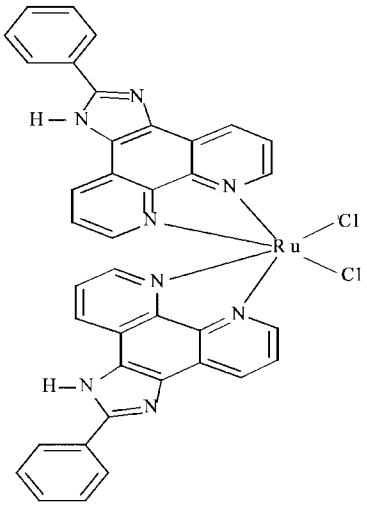
1997; Sullivan *et al*., 1978; Goss and Abruna, 1985). Purification of the compounds was monitored by thin layer chromatography and ^{1}H NMR.



$Ru(L1)_2Cl_2$: Selected IR (KBr)ν_{maks}(cm^{-1}) : 3105, 3048, 3024 (Ar C-H), 1678, 1640, 1570, 1444 (C=O, C=N, C=C), 419 (M-Cl), 327 (M-N). ^{1}H-NMR(300 MHz) (DMSO-d_6) δ (ppm): 7.60 (2H, t), 7.65 (2H, t), 9.20 (4H, dd), 9.25 (4H, dd). MS (ES): m/z: 593 [M+1]

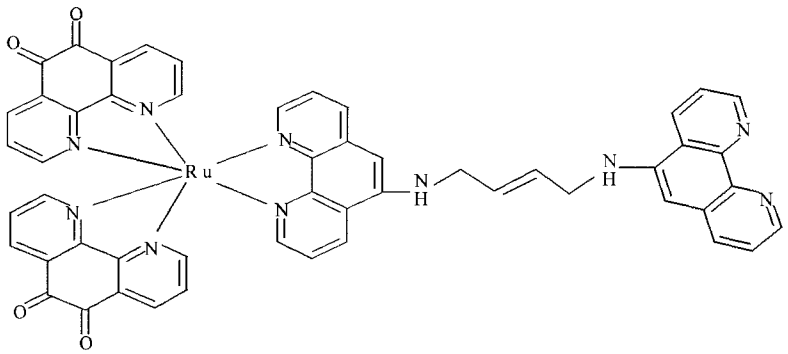


$Ru(L2)_2Cl_2$: Selected IR (KBr)ν_{maks}(cm^{-1}) : 3420, 3114, 3020 (Ar N-H, C-H), 1658, 1590, 1467 (C=N, C=C), 439 (M-Cl), 385 (M-N). ^{1}H-NMR (300 MHz) (DMSO-d_6) δ (ppm) : 7.55 (4H, t), 7.77 (2H, q), 8.10-8.35 (4H, m), 8.86 (2H, d), 10.50 (2H, d). MS (ES): m/z: 612 [M+1]

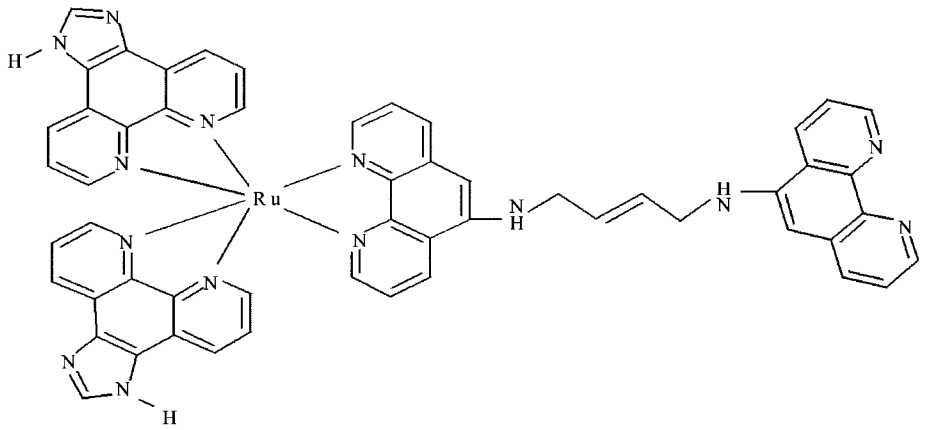


$Ru(L3)_2Cl_2$: Selected IR (Kbr)ν_{maks}(cm^{-1}) : 3450, 3070, 3016 (Ar N-H, C-H), 1628, 1601, 1447 (C=N, C=C), 417 (M-Cl), 380 (M-N). ^{1}H-NMR (300 MHz) (DMSO-d_6) δ (ppm) : 7.55 (4H, t), 7.70-7-85 (6H, m), 8.20-8.40 (8H, m), 8.50-8.56 (4H, m), 10.50 (2H, d). MS (ES): m/z: 764 [M+1]

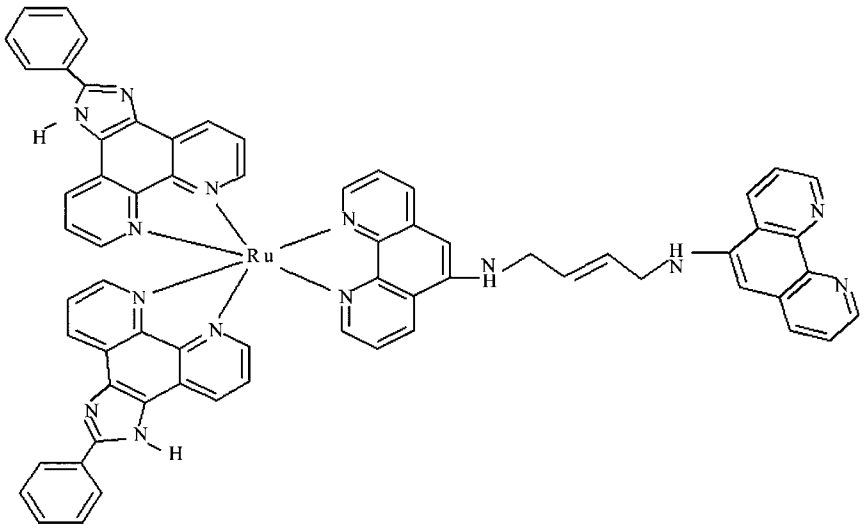
Synthesis of $[Ru(L)_2B](PF_6)_2$ complexes: $[Ru(L)_2B](PF_6)_2$ complexes was synthesized according to published methods (Sullivan *et al*., 1978; Goss and Abruna, 1985; Jing *et al*., 2000; Lecomte *et al*., 1993; Kelly *et al*., 1987). Ru $(L)_2Cl_2$ (1 mmol) and appropriate L (1 mmol) in MeOH were heated to reflux under argon for 6 h. After cooling to room temperature, MeOH was evaporated. The complex was precipitated as a dark coloured solid by the addition of saturated aqueous NH_4PF_6. The product was filtered and washed with water and diethylether. The complex was dried in vacuo for 24 h and stored in a desiccator. Recrystallization from acetonitrile/diethylether or acetone/ toluene was used for purification. The compound was characterised by IR, ^{1}H-NMR and mass spectroscopic data.



$[Ru(L1)_2B](PF_6)_2.2H_2O$: Selected IR (KBr)$\nu_{maks}(cm^{-1})$: 3420, 3140, 3107, 3076 (Ar N-H, C-H), 1621, 1624, 1446, 1378 (C=O, C=N, C=C), 290 (M-N). ^{1}H-NMR (300 MHz) (DMSO-d_6) δ (ppm) : 3.36-3.38 (4H, m), 4.07-4.15 (2H, m), 7.00-7.25 (5H, m), 7.60-8.13 (7H, m), 8.38-8.95 (8H, m), 9.07-9.23 (6H, m), 9.30 (1H, bs), 9.46 (1H, bs). MS (ES): m/z: 931 [M+1].

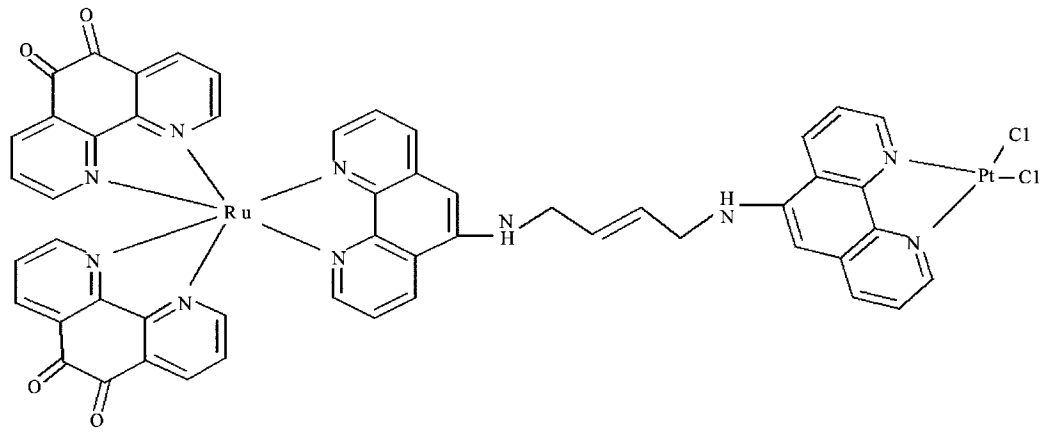


$[Ru(L2)_2B](PF_6)_2.2H_2O$: Selected IR (KBr)$\nu_{maks}(cm^{-1})$: 3420, 3140, 3107, 3076 (Ar N-H, C-H), 2988, 2945 (Al C-H), 1625, 1620, 1439 (C=N, C=C), 286 (M-N). ^{1}H-NMR (300 MHz) (DMSO-d_6) δ (ppm) : 3.38-3.40 (4H, m), 4.08-4.16 (2H, m), 7.60-8.13 (6H, m), 8.15-8.34 (12H, m), 8.58-8.75 (8H, m), 8.95-8.99 (2H, m), 9.09-9.26 (2H, m), 10.41 (1H, bs), 10.49 (1H, bs). MS (ES): m/z: 983 [M+1].

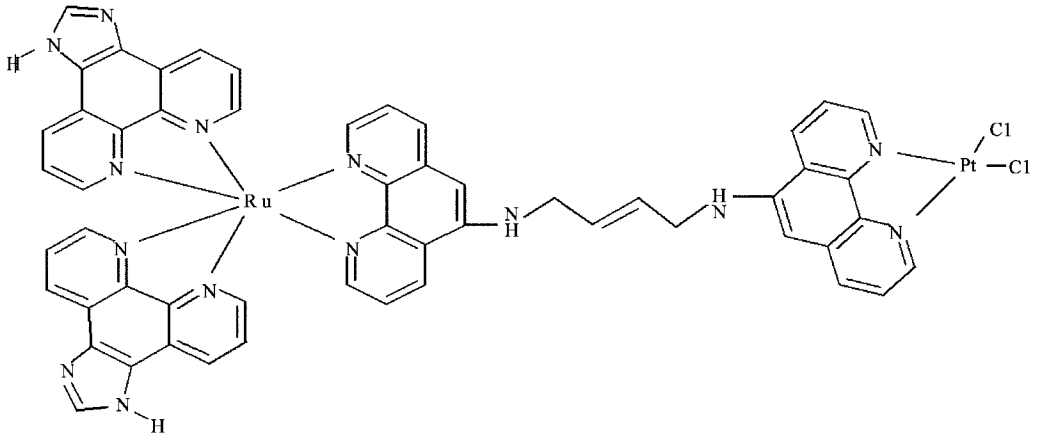


$[Ru(L3)_2B](PF_6)_2.2H_2O$: Selected IR data (KBr disk ν_{max}/cm^{-1}): 3450, 3070, 3016 (Ar N-H, C-H), 1628, 1601, 1447 (C=N, C=C), 289 (M-N). ^{1}H-NMR (300 MHz) (DMSO-d_6) δ (ppm) : 3.36-3.39 (4H, m), 4.06-4.15 (2H, m), 7.56-8.15 (10H, m), 8.30-8.55 (12H, m), 8.60-8.73 (14H, m), 8.97-9.33 (2H, m), 10.80 (1H, bs), 10.89 (1H, bs). MS (ES): m/z: 1059 [M+1].

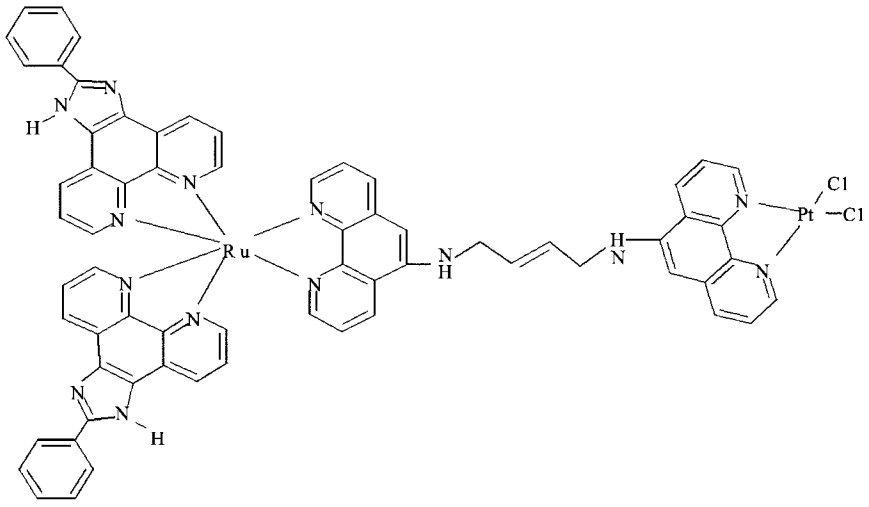
Synthesis of $[Ru(L)_2B]$ $PtCl_2$ complexes: The mixture of $[Ru(L)_2B](PF_6)_2$ solution in EtOH and K_2PtCl_4 in H_2O was stirred at room temperature for 12 h. The precipitate was filtered and washed several times with water and diethylether and dried in vacuo. The compound was characterised by IR, ^{1}H-NMR and mass spectroscopic data.



[Ru (L1)$_2$B] $PtCl_2.2H_2O$: Selected IR (KBr)ν_{maks}(cm^{-1}): 3445, 3148, 3100, 3087 (Ar N-H, C-H), 1640, 1632, 1456, 1392 (C=O, C=N, C=C), 327 (M-Cl), 287 (M-N). ^{1}H-NMR (300 MHz) (DMSO-d_6) δ(ppm) : 3.37-3.39 (4H, m), 4.17-4.23 (2H, m), 7.60-9.20 (20H, m), 9.55-9.75 (6H, m), 10.25-10.40 (2H, bm). MS (ES): m/z: 1197 [M+1]



[Ru (L2)$_2$B] $(PF_6)_2.2H_2O$: IR (KBr)ν_{maks}(cm^{-1}) : 3420, 3147, 3076 (Ar N-H, C-H), 1625, 1619, 1442 (C=N, C=C), 325 (M-Cl), 288 (M-N). ^{1}H-NMR (300 MHz) (DMSO-d_6) δ (ppm) : 3.38-3.45 (4H, m), 4.11-4.18 (2H, m), 7.68--8.54 (18H, m), 8.68-8.83 (8H, m), 9.15-9.29 (2H, m), 9.86-9.98 (2H, m), 10.55-10.67 (2H, bm). MS (ES): m/z: 1249 [M+1].



[Ru $(L3)_2B$] $PtCl_2.2H_2O$: Selected IR data (KBr disk ν_{max}/cm^{-1}): 3450, 3070, 3016 (Ar N-H, C-H), 1628, 1601, 1447 (C=N, C=C), 325 (M-Cl), 289 (M-N). ^{1}H-NMR (300 MHz) (DMSO-d_6) δ (ppm) : 3.36-3.39 (4H, m), 4.06-4.15 (2H, m), 7.65-8.24 (22H, m), 8.67-8.78 (14H, m), 9.27-9.45 (2H, m), 10.85-10.91 (2H, bm). MS (ES): m/z: 1325 [M+1].

Mutagenicity

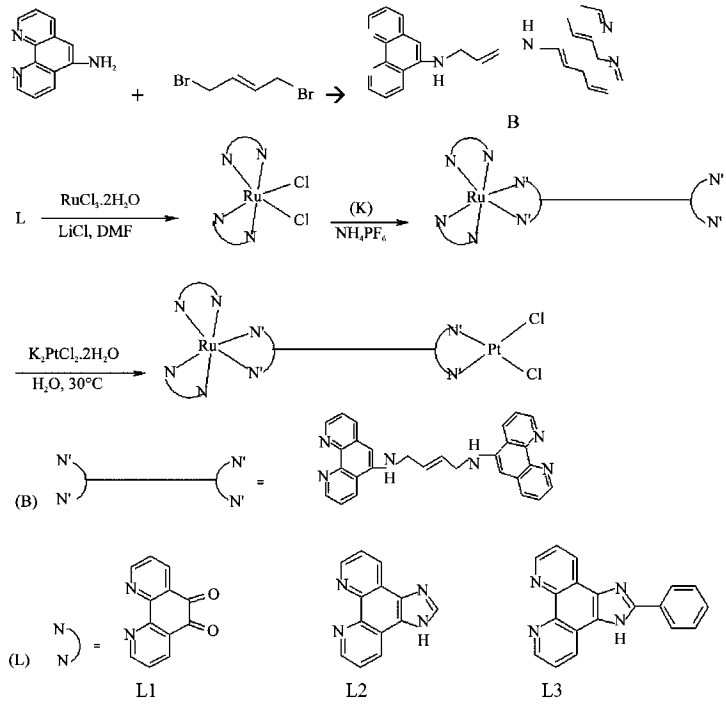
Chemicals: Dimethyl sulfoxide (DMSO), D-glucose-6-phosphate, disodium salt, L-histidine monohydrate, D-biotin, ampicillin trihydrate, 2-aminofluorene, were obtained from Sigma. Nutrient broth and nutrient agar were obtained from Oxoid, Sodium azide from Merc. Salmonella typhimurium strains TA 98 and TA100 were kindly supplied by Professor B.N. Ames (University of California, Berkeley, C.A. USA). Genotype controls and preservations of tester strains were done according to Maron and Ames (1983).

Mutagenicity assay: Ruthenium derivatives were dissolved in dimethylsulfoxide freshly for each experiment, and tested in five increasing concentrations. All experiments were done in triplicate. Mutagenicity of ruthenium derivatives was evaluated by the Salmonella/microsome assay, based on the plate-incorporation method (Maron and Ames, 1983), using *S. typhimurium* test strains TA 98 and TA 100. The test strains from frozen cultures were grown overnight at 37°C for 12-14 h in nutrient broth. Mutagenicity assay was performed by adding 0.1 mL^{-1} of the overnight bacterial culture ((1-2 x 10^8 cell mL^{-1}) and 0.1 mL^{-1} of test compounds at different concentrations (dissolved in DMSO) to the test tubes containing 2 mL top agar, vortexed a few seconds and then poured onto a plate containing minimal glucose agar. The plates were incubated at 37°C for 48 h and the his+ revertant colonies were manually counted. The positive mutagens used as positive controls in each experiment were sodium azide for TA100, 4-nitro-o-phenylenediamine(at 200 μg/plate) for TA 98 and DMSO was used as a negative (solvent) control. In all experiments the concentrations were selected on the basis of the toxicity. Toxicity was apparent either as a reduction in the number of his+ revertants, or as an alteration in the auxotrophic background (i.e. background lawn).

Statistics: The statistical analysis was performed by a Dunnett's test to compare the treated groups according to control group.

RESULTS AND DISCUSSION

In the current work, we described the synthesis and characterization of some Ru (III) and Pt (II) dinuclear complexes. 1,10-phenanthroline-5,6-dione (dp), imidazo



Scheme 1: The synthesis of L and its metal complexes

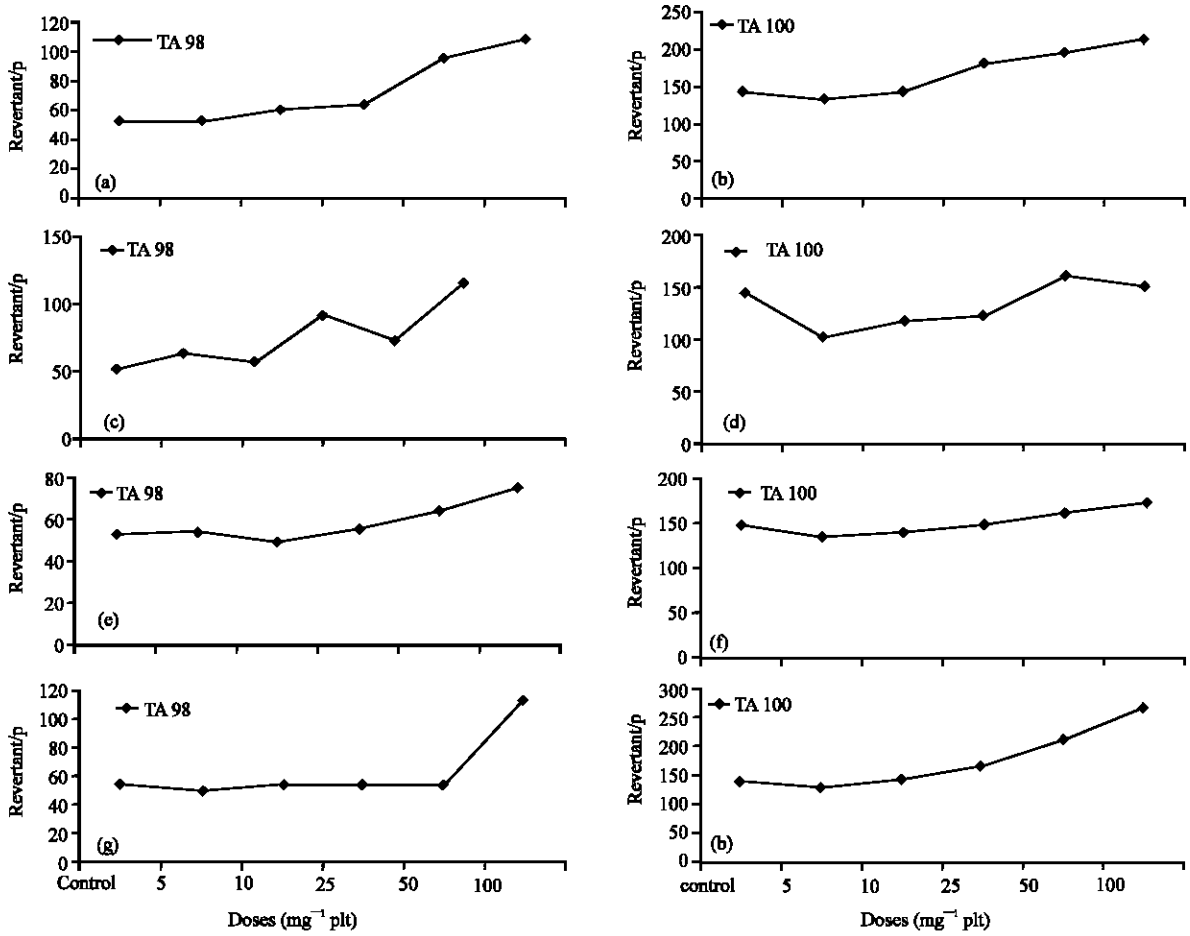


Fig. 1: (a) Mutagenicity of RuL_2BPt in the *S. typhimurium* TA98 tester strain without metabolic activation. (b) Mutagenicity of RuL_2BPt in the *S. typhimurium* TA100. (c) Mutagenicity of RuL_3BPt in the *S. typhimurium* TA98. (d) Mutagenicity of RuL_3BPt in the *S. typhimurium* TA100. (e) Mutagenicity of RuL_4BPt in the *S. typhimurium* TA98. (f) Mutagenicity of RuL_4BPt in the *S. typhimurium* TA100. (g) Mutagenicity of cis-platin in the *S. typhimurium* TA98. (h) Mutagenicity of cis-platin in the *S. typhimurium* TA100 (Dunnett's test, $p<0.05$).

[4, 5-f] [1,10] phenanthroline (ip) and 2-phenylimidazo [4, 5-f] [1,10] phenanthroline (pip) were used as ligands for $[Ru(L)_2(B)]PtCl_2$ complexes. The structures of the compounds obtained were elucidated by spectral data.

IR spectra of the ligands showed two bands between 3127-2786 cm^{-1} due to v (N-H), 1654-1635 cm^{-1} due to v (C=N) which are different from the spectrum of ruthenium (II) complexes. By comparison of the spectra of the metal complexes with those of phen-dione and phenanthroline, imidazophenanthroline or phenylimidazophenanthroline, it was determined that the bands centered at about 1700 and 1600 cm^{-1} are carbonyl stretches on the phen-dione ligand. In general, the carbonyl stretches are relatively insensitive to changes in the metal center and its coordination environment on the carbonyl stretch is a secondary effect.

In the ^{1}H-NMR spectra, the peaks of the Ru (II) complexes' aromatic protons were observed at about 6.54-10.12 ppm as multiplets. Due to greater aromatic planar and stronger deshielding effect, the phenanthroline protons show large downfield shifts. In addition, the proton resonance on the nitrogen atom of the imidazole rings of ip and pip were not observed, because the proton is an active proton and exchanges quickly between the two nitrogens of the imidazole rings. A similar example has been reported in the literature (Jiang *et al*., 2001; Wu *et al*., 1997). Since there is a higher content of N atoms with stronger electronegativity in the intercalated ligand ip or pip than in the ancillary ligand phen, the protons in the intercalated ligand have more electron-deficient characteristics than those in the ancillary ligand. Elemental analyses and especially M+1 values in ES/MS spectra were as expected.

Ames Test system was used in order to identify the most significant sites for an initial assessment of the basin as to the presence of mutagenic compounds (Vargas *et al*., 2001). Mutagenicity of the ruthenium derivatives and cis-platin are displayed in Fig. 1. In order to show the mutagenic potential of ruthenium Compounds according to cis-platin by using *Salmonella* Strains of TA 98 and TA 100.

Results were evaluated: The Fig. 1 shows the number of revertant colonies obtained for each concentration of the ruthenium derivatives. Results were considered positive if there was a dose-related two fold increase in the number of revertant colonies.

The results of mutagenicity assay of ruthenium derivatives were given in Fig. 1a to F. those for of cis-platin displayed in Fig. 1. G-H. Toxicity was determined as to presence of significantly reduced number of revertants, compared to solvent control in TA 100 (Zeytinoglu *et al*., 2000). Doses of ruthenium derivatives and cis-platin in excess of 100 μg/plt were toxic therefore higher doses were not used in the mutagenicity assay. 20 μg/plate 4-nitro-o-phenylenediamine (NPD) and 5 μg/plate sodium azide (AZS) were used as a positive control.

RuL_2BPt complex on TA98 *Salmonella* strain was found mutagenically effective at the dose of 100 μg/plt. The lower doses were not found mutagenic because the number of the revertant colonies did not exceed the spontaneous control colonies (Fig. 1a). RuL_2BPt at the doses of 100 μg/plate on TA 100 strain of *Salmonella* was found slightly mutagenic (Fig. 1b).

RuL_3BPt complex gave mutagenic response on TA98 strain at dose of 100 μg/plt (Fig. 1c). On the other hand none of the doses of RuL_3BPt did not exhibit any mutagenicity on S*almonella typhimurium* strain TA 100 (Fig. 1d).

RuL_4BPt gave dose related increase in revertant on both strains of *Salmonella* strains. None of the doses of the chemical showed any mutagenic effect on TA98 and TA100 strains (Fig. 1e,f)

Ruthenium derivatives have been proposed as potential antitumor activity (Grguric-Sipka *et al*., 2003). In vitro studies have been confirming the binding of Ru compounds to the DNA and inhibit the transcription by stabilization of the DNA duplex structure, cytotoxic activity tests in cultured cells indicate a direct correlation between this activity (Clarke, 2003).

There was no significant increase of revertants due to the treatment with the doses of 5, 10, 25, 50 μg/plt cis-platin on TA98. 100 μg/plt dose showed mutagenic effect on TA98 (Fig. 1g). On TA100 strain dose related increase in the number of revertant colonies was observed but the number of revertants were not double the number of spontaneous colonies up to 100 μg/plt dose. Only 100 μg/plt dose showed mutagenic effect (Fig. 1h).

ACKNOWLEDGMENTS

The authors are grateful to Anadolu University, Commission of Scientific Research Projects, for financial support (Project No: 030328).

REFERENCES

Alp, M., H. Goker, R. Brun and S. Yildiz, 2009. Synthesis and antiparasitic and antifungal evaluation of 2'-arylsubstituted-1H,1'H- [2,5'] bisbenzimidazolyl-5-carboxamidines. Eur. J. Med. Chem., 44: 2002-2008.

Ambroise, A. and B.G. Maiya, 2000. Ruthenium (II) complexes of 6,7 dicyanodipyridoquinoxaline: Synthesis, luminescence studies and DNA interaction. Inorg. Chem., 39: 4264-4272.

Arounagiri, S., D. Easwaramoorthy, A. Ashokkumar, A. Dattagupta and B.G. Maiya, 2000. Cobal t (III), nickel (II) and ruthenium (II) complexes of 1,10-phenanthroline family of ligands: DNA binding and photocleavage studies. J. Chem. Sci., 112: 1-17.

Barton, J.K. and E. Lolis, 1985. Chiral discrimination in the covalent binding of Bis (phenanthroline) dichlororuthenium (II) to B-DNA. J. Am. Chem. Soc., 107: 708-709.

Benkli, K., Y. Tunali, Z. Canturk, O. Artagan and F. Alanyali, 2009. Cytotoxic and genotoxic effects of $[Ru(phi)_3]^{2+}$ evaluated by Ames/*Salmonella* and MTT methods. Eur. J. Med. Chem., 44: 2601-2605.

Chao, H., W.J. Mei, Q.W. Huang and L.N. Ji, 2002. DNA binding studies of ruthenium(II) complexes containing asymmetric tridentate ligands. J. Inorg. Biochem., 92: 165-170.

Chen, A.Y., C. Yu, A.L. Bodley, L.F. Peng and L.F. Liu, 1993. A new mammalian DNA topoisomerase I poison Hoechst 33342: Cytotoxicity and drug resistance in human cell cultures. Cancer Res., 53: 1332-1337.

Clarke, M.J., 2002. Ruthenium metallopharmaceuticals. Coord. Chem. Rev., 232: 69-93.

Clarke, M.J., 2003. Ruthenium metallopharmaceuticals. Coord. Chem. Rev., 236: 209-233.

Collins, J.G., J.R. Aldrich-Wright, L.D. Greguric and P.A. Pellegrini, 1999. Binding of the delta and lambda-enantiomers of [Ru (dmphen) (2) dpq] (2+) to the hexanucleotide d (GTCGAC)(2). Inorg. Chem., 38: 5502-5509.

Dandliker, P.J., R.E. Holmlin and J.K. Barton, 1997. Oxidative thymine dimer repair in the DNA helix. Science, 275: 1465-1468.

Deng, H., J. Li, K.C. Zheng, Y. Yang, H. Chao and L.N. Ji, 2005. Synthesis, c haracterization, structures and DNA-binding properties of complexes $[Ru(bpy)_2(L)]^{2+}$ (L = ptdb, ptda and ptdp) with asymmetric intercalative ligands. Inorg. Chim. Acta., 358: 3430-3440.

Devran, P.B., 1986. Design of sequence-specific DNA-binding molecules. Science, 232: 464-471.

Farrell, N., 2002. Biomedical uses and applications of inorganic chemistry: An review. Coord. Chem. Rev., 232: 1-4.

Garoufis, A., J.G. Liu, L.N. Ji and N. Hadjiliadis, 2003. Enantioselective binding of Λ- and Λ-$[Ru(bpy)_2(HPIP)]Cl_2$ (HPIP = 2-(2-hydroxyphenyl)imidazo[4,5-f][1,10]phenanthroline) to the hexanucleotide $[d(5'\text{-}GTCGAC\text{-}3')_2]$. J. Inorg. Biochem., 93: 221-234.

Goss, C.R. and H.D. Abruna, 1985. Spectral, electrochemical and electrocatalytic properties of phenanthroline 5, 6 dione complexes of transition metals. Inorg. Chem., 24: 4263-4267.

Gray, H.B. and J.R. Winkler, 1996. Electron transfer in proteins. Ann. Rev. Biochem., 65: 537-561.

Grguric-Sipka, S.R., R.A. Vilaplana, J.M. Perez, M.A. Fuertes and C. Alonso *et al*., 2003. Synthesis, characterization, interaction with DNA and cytotoxicity of the new potential antitumour drug cis-K[Ru(eddp)Cl_2)]. J. Inorg. Biochem., 97: 215-220.

Holmlin, R.E., J.A. Yao and J.K. Barton, 1999. Dipyridophenazine complexes of Os (II) and red-emitting DNA probes: Synthesis, characterization and photophysical properties. Inorg. Chem., 38: 174-189.

Jackson, B.A. and J.K. Barton, 2000. Recognition of base mismatches in DNA by 5,6-chrysenequinone diimine complexes of rhodium(III): A proposed mechanism for preferential binding in destabilized regions of the double helix. Biochemistry, 39: 6176-6182.

Jing, B., T. Wu, C. Tian, M. Zhang and T. Shen, 2000. pH-dependent luminescence of ruthenium(II) polypyridine complexes. Bull. Chem. Soc. Jpn., 73: 1749-1755.

Jiang, C.W., H. Chao, R.H. Li, H. Li and L.N. Ji, 2001. Syntheses, characterization and third-order nonlinear optical properties of ruthenium(II) complexes containing 2-phenylimidazo-[4,5-f][1,10] phenanthroline and extended diimine ligands. Polyhedron, 20: 2187-2193.

Kelly, J.M., D.J. McConnell, C. OhUigin, A.B. Tossi, A. Kirsch-De Mesmaeker, A. Masschelein and J. Nasielski, 1987. Ruthenium polypyridyl complexes: Their interaction with DNA and their role as sensitisers for its photocleavage. J. Chem. Soc. Chem. Commun., 24: 1821-1823.

Kelly, S.O., N.M. Jackson, M.G. Hill and J.K. Barton, 1999. Long range electron transfer through DNA films. Angew. Chem. Int. Ed., 38: 941-945.

Komeda, S., M. Lutz, A.L. Spek, M. Chikuma and J. Reedijk, 2000. New antitumor-active azole-bridged dinuclear platinum(II) complexes: SYNTHESIS, characterization, crystal structures and cytotoxic studies. Inorg. Chem., 39: 4230-4236.

Lecomte, J.P., A.K.D. Mesmaeker, M. Demeunynck and J. Lhomme, 1993. Synthesis and characterisation of a new DNA-binding bifunctional ruthenium (II) complex. J. Chem. Soc. Faraday Trans., 89: 3261-3269.

Loehrer, P.J. and L.H. Einhorn, 1984. Cisplatin. Ann. Int. Med., 100: 704-713.

Maheswari, P.U. and M. Palaniandavar, 2004. DNA binding and cleavage properties of certain tetramine ruthenium(II) complexes of modified 1,10-phenanthrolines-effect of hydrogen-bonding on DNA-binding affinity. J. Inorg. Biochem., 98: 219-230.

March, J., 1985. Advanced Organic Chemistry, Reactions, Mechanisms and Structure. 3rd Edn., John Wiley and Sons, New York.

Maron, D.M. and B.N. Ames, 1983. Revised method for the *Salmonella* mutagenicity test. Mutat. Res., 113: 173-215.

Messori, L., F.G. Vilchez, R. Vilaplana, F. Piccioli, E. Alessio and B. Kepler, 2000a. Binding of antitumor ruthenium(III) complexes to plasma proteins. Met. Based Drugs, 7: 335-342.

Messori, L., P. Orioli, D. Vullo, E. Alessio and E. Iengo, 2000b. A spectroscopic study of the reaction of NAMI, a novel ruthenium (III) anti-neoplastic complex, with bovine serum albumin. Eur. J. Biochem., 267: 1206-1213.

Mock, C., I. Puscasu, M.J. Rauterkus, G. Tallen, J.E.A. Wolff and B. Krebs, 2001. Novel Pt(II) anticancer agents and their Pd(II) analogues: Syntheses, crystal structures, reactions with nucleobases and cytotoxicities. Inorg. Chim. Acta., 319: 109-116.

Mortelmans, K. and E. Zeiger, 2000. The Ames Salmonella/microsome mutagenicity assay. Mutat. Res., 455: 29-60.

Novakova, O., J. Kasparkove, O. Vrana, P.M. van Vliet, J. Reedijk and V. Brabec, 1995. Correlation between cytotoxicity and DNA binding of polypyridyl ruthenium complexes. Biochemistry, 34: 12369-12378.

Ortmans, L., C. Moucheron and A.K. De Mesmaeker, 1998. Ru(II) polypyridine complexes with a high oxidation power. Comparison between their photoelectrochemistry with transparent SnO2 and their photochemistry with desoxyribonucleic acids. Coord. Chem. Rev., 168: 233-271.

Patel, K.K., E.A. Plummer, M. Darwish, A. Rodger and M.J. Hannon, 2002. Aryl substituted ruthenium bisterpyridine complexes: Intercalation and groove binding with DNA. J. Inorg. Biochem., 91: 220-229.

Paw, W. and R. Eisenberg, 1997. Synthesis, characterization and spectroscopy of dipyridocatecholate complexes of platinum. Inorg. Chem., 36: 2287-2293.

Pinedo, H.M. and J.M. Schornagel, 1996. Platinum and other Metal Coordination Compounds in Cancer Chemotherapy. Plenum Press, New York.

Pyle, A. and J.K. Barton, 1990. Probing nucleic acids with transition metal complexes. Prog. Inorg. Chem., 38: 413-475.

Qu, X. and J.B. Chaires, 2001. Hydration changes for DNA intercalation reactions. J. Am. Chem. Soc., 123: 1-7.

Reedijk, J., 2003. New clues for platinum antitumor chemistry: Kinetically controlled metal binding to DNA. Proc. Natl. Acad. Sci., 100: 3611-3616.

Rodriguez-Bailey, V.M., K.J. LaChance-Galang, P.E. Doan and M.J. Clarke, 1997. ^{1}H and ^{31}P NMR and EPR of pentaammineruthenium(III) complexes of endocyclically coordinated nucleotides, nucleosides and related heterocyclic bases. Autoxidation of [(Guoκ^{N7})$(NH_3)_5Ru^{III}$] (Guo = Guanosine). crystal structure of [7MeGua?^{N9} $(NH_3)_5$Ru]$Cl_3 \cdot 3H_2O$. Inorg. Chem., 36: 1873-1883.

Sitlani, A., E.C. Long, A.M. Pyle and J.K. Barton, 1992. DNA photocleavage by phenanthrenequinone diimine complexes of rhodium (III): Shape-selective recognition and reaction. J. Am. Chem. Soc., 114: 2303-2312.

Stemp, D.E., R.E. Holmlin and J.K. Barton, 2000. Electron transfer between metal complexes bound to DNA: Variations in sequence, donor and metal binding mode. Inorg. Chim. Acta., 297: 88-97.

Sullivan, B.P., D.J. Salmon and T.J. Meyer, 1978. Mixed phosphine 2,2'-bipyridine complexes of ruthenium. Inorg. Chem., 17: 3334-3341.

Tuite, E., 1998. Coordination Complexes and Nucleic Acids, Perspectives on Electron Transfer, Binding Mode and Cooperativity. In: Organic and Inorganic Photochemistry, Ramamurthy, V. and K.S. Schanze (Eds.). Vol. 2, Marcel Dekker, New York, pp: 55-74.

Vaidyanathan, V.G. and B.U. Nair, 2002. Synthesis, characterization and DNA binding studies of a ruthenium(II) complex. J. Inorg. Biochem., 91: 405-412.

Vargas, V.M.F., S.B. Migliavacca, A.C. Melo, R.C. Horn, R.R. Guidobono, I.C.F. Sa-Ferreira and M.H.D. Pestana, 2001. Genotoxicity assessment in aquatic environments under the influence of heavy metals and organic contaminants. Mutat. Res., 490: 141-158.

Wang, J.C., 1985. DNA Topoisomerases. Annu. Rev. Biochem., 54: 665-697.

Waters, M.D., A.L. Brady, H.F. Stack and H.E. Brockman, 1990. Antimutagenicity profiles for some model compounds. Mutat. Res., 238: 57-85.

Watt, T.A., C. Tong, A.P. Arnold and J.G. Collins, 1996. The selective B-A conformational transition of the central dinucleotide (CpG) segment of d(CAATCCGGATTG)2 induced by delta-Co(en) 3 (3+). Biochem. Mol. Biol. Int., 38: 383-391.

Wu, J.Z., B.H. Ye, L. Wang, L.N. Ji, J.Y. Zhou, R.H. Li and Z.Y. Zhou, 1997a. Bis (2,2-bipyridine)ruthenium (II) complexes with imidazo [4,5-f] [1,10]-phenanthroline or 2-phenylimidazo [4,5-f] [1,10] phenanthroline. J. Chem. Soc. Dalton Trans., 119: 1395-1401.

Wu, J.Z., L. Li, T.X. Zeng, L.N. Ji, J.Y. Zhou, T. Lou and R.H. Li, 1997b. Synthesis, characterization and luminiscent DNA-binding study of a series of ruthenium complexes containing 2-arylimidazo[f]1,10-phenanthroline. Polyhedron, 16: 103-107.

Xiong, Y., X.H. Zou, J.Z. Wu, L.N. Ji, R.H. Li, J.Y. Zhou and K.B. Yu, 1999. Synthesis, structure and DNA-binding studies on the 2-(3-chlorophenyl)imidazo[4,5-f]1,10-phenanthroline-bis(2,2'-bipyridine)-ruthenium (II) complex. Trans. Meter. Chem., 24: 263-267.

Yamada, M., Y. Tanaka, Y. Yoshimato, S. Kuroda and I. Shimao, 1992. Synthesis and properties of diamino-substituted dipyrido [3,2-a: 2',3'-c]phenazine. Bull. Chem. Soc. Jpn., 65: 1006-1011.

Zeytinoglu, H., E. Ergene and B. Tuylu, 2000. Mutagenicity assay in *Salmonella* for thirteen 2-substitued-1-H-phenanthro imidazoles. Drug Chem. Toxicol., 26: 245-257.

Zhen, Q.X., Q.L. Zhang, J.G. Liu, B.H. Ye, L.N. Ji and L. Wang, 2000. Synthesis, characterization and DNA binding of ruthenium(II) complexes containing the atatp ligand. J. Inorg. Biochem., 78: 293-298.

Zimmer, C.H. and U. Wahnert, 1986. Nonintercalating DNA-binding ligands: Specificity of the interaction and their use as tools in biophysical, biochemical and biological investigations of the genetic material. Prog. Biophys. Mol. Biol., 47: 31-112.