Coordination Chemistry of Tropolone-Based Antimitotic Drugs and the Antineoplastic Behavior of Some Ruthenium(II) and Platinum(II) Derivatives

Jeffrey B. Helms, Ling Huang, Reyn Price, B. Patrick Sullivan,* and Beverly A. Sullivan*

Departments of Pharmacy and Chemistry, University of Wyoming, Laramie, Wyoming 82071-3838

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Aspects of the coordination chemistry of the antimitotics colchicine (Col) and trimethylcolchicinic acid (Tmca) with the metal fragments $Pt(bpy)^{2+}$ and $Ru(bpy)_2^{2+}$ are reported. In addition, the coordination chemistry of the tropolone anion (Tp) with the same fragments, which serves as a model for one mode of binding of the antimitotic, is described. The new complexes have been characterized by a variety of spectroscopic techniques. We have tested the *in vitro* antitumor activity of the new complexes against human chronic myelogenous leukemia, K562, and human colon adenocarcinoma, COLO 205. In these experiments we can demonstrate cytotoxicity comparable to that of cisplatin, and surprisingly, activity of the substitutionally-inert $Ru(bpy)_2^{2+}$ complexes of Tmca and Col is comparable to that of colchicine itself. It is suggested that the antineoplastic activity is predominantly due to the *intact* complexes since solution studies of all the Ru and Pt complexes at room temperature in 0.1 M saline/5% DMSO show no degradation products over 48 h.

Introduction

Metal-containing compounds are among the most prescribed anticancer drugs. Of these, *cis*-dichlorodiammine platinum(II) (cisplatin, $(NH_3)_2PtCl_2$) is clinically the most important.[1-6] Cisplatin is active against testicular and head and neck cancers[4] but is relatively inactive against major cancers such as colon and breast. In addition to a limited spectrum of activity, cisplatin is associated with a number of deleterious side effects such as nephrotoxicity, neurotoxicity, and severe emesis.[3,4] Due to cisplatin's inherent activity, but unfortunate drawbacks, a major research thrust has been the search for new derivatives with greater activity and/or decreased toxicity.[2,3] These studies have led to such compounds as carboplatin and iproplatin which have a different activity spectrum but are only limited advances in the search for improved platinum containing anticancer drugs.[3,4]

Improvement in the selectivity and/or activity of metal-containing drugs might be possible through a "dual function" approach. By dual function we mean those compounds that combine two potential biological mechanisms in a single molecule. Such substances could be greater than the sum of their parts in that they offer greater activity toward specific tumors or be less than the sum by mitigating the severe toxicity of one function.

A class of effective anticancer agents, which could serve as building blocks for dual function drugs, and which are synthetically amenable to coordination to metal centers like platinum, are the tropolone-based antimitotic drugs such as colchicine (Col) or trimethylcolchicinic acid (Tmca). By itself, Col is a potent anticancer agent,[10,11] but its clinical usefulness is limited by severe toxicities.[10,12,13] Trimethylcolchicinic acid, a derivative of colchicine, is an active, less toxic antitumor agent[10,14] that has been evaluated in Phase I clinical trials.[10] Figure 1 shows the structures of tropolone, Col, and Tmca. Tropolone (TpH) derivatives with simple substitution patterns have also been shown to possess *in vitro* anti cancer properties.[15] It has been found that colchicine and many of its derivatives bind strongly to the tubulin protein,[16,17] and this may be a basis for antineoplastic behavior via interrupted mitosis.

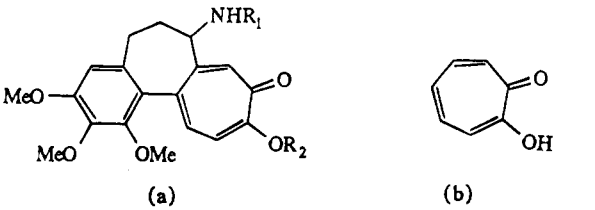

Figure 1. Structures of (a) colchicine when $R_1 = C(O)CH_3$ and $R_2 = CH_3$ and trimethylcolchicinic acid when $R_1 = H$ and $R_2 = H$, and (b) tropolone.

In this paper we report on aspects of the coordination chemistry of the antimitotics Col and Tmca with the metal fragments $Pt(bpy)^{2+}$ and $Ru(bpy)_2^{2+}$. In addition, the coordination chemistry of the tropolone anion (Tp), which serves as a model for one mode of binding of the antimitotic is described. An aspect that this chemistry touches upon is the binding of natural products like Col to transition metal centers to achieve

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biological effects. The new complexes have been characterized by a variety of spectroscopic techniques. Further, we have tested their *in vitro* antitumor activity against human chronic myelogenous leukemia, K562, and human colon adenocarcinoma, COLO 205. In these experiments we can demonstrate activity comparable to that of cisplatin and, surprisingly, activity of the substitutionally-inert $Ru(bpy)_2^{2+}$ complexes of Tmca and Col that is comparable to that of colchicine itself. A notable aspect of our studies is the use of human tumor cell models.

Experimental Section

Materials. $(bpy)_2RuCl_2 \cdot 2H_2O$[18] was prepared by the published procedure. Tropolone (TpH) and 97% colchicine were purchased from Aldrich Chemical Co. and used as received. Trimethylcolchicinic acid, trichloroacetic acid, sulforhodamine B and cisplatin were purchased from Sigma Chemical Co. All the chemicals were used without further purification. The biologicals, RPMI 1640 with L-glutamine, fetal or newborn calf serum, penicillin/streptomycin solution, and 0.4% trypan blue also were purchased from Sigma. Human chronic myelogenous leukemia cells (K562) and human colon adenocarcinoma (COLO 205) were purchased from American Type Culture Collection (ATCC), Rockville, MD.

Preparation of Complexes. (bpy)$PtCl_2$. To 25 mL of toluene were added 100 mg of $(COD)PtCl_2$ and 50 mg of bpy. The mixture was heated at reflux under a blanket of dry nitrogen for 8 h after which time the yellow microcrystalline solid was filtered, washed with fresh toluene, followed by diethyl ether, and air-dried. The properties of the complex were identical to that prepared by the conventional route.[19]

$(bpy)_2Ru(Tp)(PF_6)$. $(bpy)_2RuCl_2 \cdot 2H_2O$ (0.93 mmol, 484 mg) and TpH (2 mmol, 244 mg) were mixed in 40 mL of 95% ethanol. The mixture was heated at reflux with magnetic stirring under nitrogen for 2 h until most of the dark purple solid $(bpy)_2RuCl_2$ had disappeared. The solution was filtered, and 10 mL of aqueous NH_4PF_6 was added to the filtered solution. A black crystalline solid (needles) was collected, washed with ethanol and ether and air-dried. Yield: 491 mg (93%). Crystals suitable for crystallographic study were obtained by slow diffusion of ether into an acetonitrile solution of the complex. Anal. Calcd for $C_{17}H_{13}N_5O_2F_6PRu$: C, 47.72; H, 3.11; N, 8.18. Found: C, 47.51; H, 3.18; N, 8.18. Proton NMR in CD_3CN (ppm): 6.78 (1H, M); 7.09−7.12 (4H, M); 7.20, 7.23, 7.25 (2H, T); 7.60, 7.62, 7.63 (2H, T); 7.68, 7.70 (2H, D), 7.73, 7.75, 7.77 (2H, T); 8.05, 8.05, 8.09 (2H, T); 8.34, 8.36 (2H, D); 8.48, 8.49 (2H, D); 8.75, 8.76 (2H, D).

$(bpy)_2Ru(Tmca^NH)(PF_6)_2$. (The notation $Tmca^NH$ refers to an amine protonated ligand; see text.) $(bpy)_2RuCl_2$ (0.28 mmol, 145 mg) and Tmca (0.33 mmol, 113 mg) were combined in 95% ethanol. The mixture was heated at reflux under nitrogen for 24 h. After this time the solution was filtered, and aqueous NH_4PF_6 was added to the filtered solution. The complex was isolated as the PF_6^- salt by filtration. After air-drying the crude complex was purified by aluminum oxide chromatography using toluene/CH_3CN mixtures.[18] Yield: 102 mg (35%). Anal. Calcd for $C_{39}H_{37}N_5O_5F_{12}P_2Ru$: C, 44.75; H, 3.56; N, 6.69. Found: C, 45.01; H, 3.75; N, 6.58. Proton NMR in CD_3CN (ppm): 2.46 (1H, M); 2.30 (1H, M); 2.12 (1H, M); 3.58, 3.62 (3H,2S); 3.85, 3.86 (6H, 2D); 4.30, 4.40 (1H, 2M); 6.12, 6.25 (1H, 2D); 6.55−8.80 (20H, many peaks).

$(bpy)_2Ru(DMCol)(PF_6)$. (The notation DMCol refers to a demethylated Col ligand; see text.) Colchicine (0.5 mmol, 200 mg) and sodium acetate (175 mg, 2 mmol) were dissolved in 60 mL of methanol and water (2:1 by volume). $(bpy)_2RuCl_2$ (0.47 mmol, 242 mg) was added to the solution, and the mixture was heated at reflux under nitrogen for 3 days. Aqueous NH_4PF_6 was employed to isolate the complex. After air-drying a black-red complex was obtained and purified by aluminum oxide chromatography as described above. Yield: 145 mg (33%). Anal. Calcd for $C_{41}H_{38}N_5O_6F_6PRu$: C, 52.23; H, 4.06; N, 7.43. Found: C, 52.47; H, 4.05; N, 7.40. Proton NMR in CD_3CN (ppm): 1.78, 1.92 (3H, 2S); 2.15 (1H, M); 2.25 (1H, M); 2.45 (1H, M); 3.50, 3.52 (3H, 2S); 3.86, 3.87 (6H, 2D); 4.30, 4.41 (1H, 2D); 5.96, 6.09 (1H, 2D); 6.53−8.87 (20H, many peaks). ^{13}C NMR in CH_3CN (ppm): 22.86 (D, $NHCOCH_3$); 30.27; 37.60 (M); 54.85 (M); 56.66; 61.28; 61.78; 108.09; 123.47; 123.89; 126.38; 127.30; 136.27; 138.12 (D); 153.12−158.40 (M); 159.41; 160.81; 170.07 (D, $NHCOCH_3$)[d].

(bpy)Pt(Tp)(PF_6)-1/3CH_3CN: (bpy)$PtCl_2$ (0.24 mmol, 100 mg) and TpH (0.3 mmol, 36 mg) were combined in 40 mL of distilled water. The bright yellow mixture was heated at reflux under nitrogen for 20 h. A clear orange solution was obtained. Aqueous NH_4PF_6 was added to the filtered solution to give a fine yellow to yellow-orange precipitate. The crude product was recrystallized from acetonitrile and ether. Yield: 87 mg (60%). Anal. Calcd for one molecule of $C_{17}H_{13}N_2O_2F_6$-PPt and one-third molecule of CH_3CN in the solid: C, 33.63; H, 2.24; N, 5.18. Found: C, 33.81; H, 2.23; N, 5.09. Proton NMR in CD_3CN (ppm): 7.49−7.61 (3H, M); 7.68, 7.70, 7.73 (2H, T); 7.82, 7.86, 7.90 (2H, T); 8.15, 8.18 (2H, D); 8.26, 8.28, 8.30 (2H, T); 8.83, 8.85 (2H, D).

(bpy)Pt$(Tmca^NH)(PF_6)_2 \cdot {}^1/_2CH_3CN$. Prepared in the same way as (bpy)Pt(Tp)(PF_6) using 100 mg of (bpy)PCl_2 and 105 mg (0.3 mmol) of Tmca. Yield: 129 mg (55%). Anal. Calcd for one molecule of complex $C_{29}H_{32}N_3O_5F_{12}P_2Pt$ and one-half molecule of CH_3CN: C, 36.20; H, 3.43; N, 5.45. Found: C, 34.83; H, 3.58; N, 5.21. Proton NMR in CD_3CN (ppm): 9.02−7.20 (11H, many peaks); 6.82 (1H, S); 3.95 (3H, S); 3.88 (3H, S); 3.65 (3H, S); 3.20 (2H, M); 2.72 (3H, overlapping multiplets).

Measurements. Electrochemical data were obtained by cyclic voltammetry with a Princeton Applied Research 173/175 electrochemical apparatus along with a Hewlett-Packard 7015 B XY recorder. A platinum-bead working electrode, platinum-wire auxiliary electrode, and silver wire were used. Spectrograde acetonitrile was used with 0.1 M tetra-*n*-butylammonium hexafluorophosphate (TBAH) as supporting electrolyte. The measurements were obtained in a drybox filled with nitrogen. Calculation of the $E_{1/2}$ value was made by averaging the potential values for the highest current response on the anodic and cathodic sweeps which were carried out at a scan rate of 100 mV/s.

UV−visible spectra were measured using either a Perkin-Elmer $\lambda 9$ recording spectrophotometer or HP 8452 diode array. Quantitative measurements were made by using 1 cm quartz cells with spectrograde acetonitrile as solvent. Spectroelectrochemistry was measured using a "home-built" cell which allowed us to record UV−visible and CV data at the same time.

IR spectra were recorded on a Perkin-Elmer 1600 Series FTIR spectrometer in the range 800−4000 cm^{-1} with a resolution of 4.0 cm^{-1}.

Proton and ^{13}C NMR spectral data were acquired with JEOL NMR spectrometers at either 270 or 400 MHz. Both proton and ^{13}C NMR spectral shift data were reported vs the deuterated solvent as internal standard and referenced to TMS.

Elemental analyses (C, H, N) were provided by Desert Analytics Organic Microanalysis, Tuscon, AZ 85717-9990.

Cell Line and Culture Conditions. Mycoplasma free human chronic myelogenous leukemia, K562 (ATCC No. CCL 243) and human colon adenocarcinoma, COLO 205 (ATCC CCL 222), were obtained from the American Type Culture Collection (ATCC) (Rockville, MD). The cell lines were maintained in RPMI 1640 with L-glutamine, penicillin/streptomycin, and 10% newborn or 5% fetal calf serum. The cells were cultured in 75 and 250 mL Falcon tissue culture flasks in a water-saturated atmosphere of 95% air and 5% carbon dioxide at 36 °C. Continuous cell lines were split and fed with fresh media every 48−72 h (depending on growth characteristics) to maintain cell concentrations of $(0.5-2) \times 10^6$ cells/mL and the pH of the media between 7.0 and 7.4. Cell counts and viabilities were ascertained utilizing trypan blue dye exclusion [20−23] visualized by a light microscope.

Cytotoxicity Assay Controls. Cisplatin and colchicine were used as positive controls. RPMI media with 2% or 6% DMSO and tropolone (0.01, 0.1 and 1 μM) served as negative controls.

Cytotoxicity Assay. Experimental drugs were dissolved in a minimum of DMSO. This solution was then diluted to 10 mL with media to give a 1 mM solution. This solution was then serially diluted with media to give solutions of the desired concentrations (100, 10, 1, 0.1 μM). A DMSO/media control of 2% DMSO−6% DMSO depending on which drugs were being used was prepared. All solutions were sterilized via passage through a 0.22 μm sterile filter.

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Approximately 25,000 K562 and COLO 205 cells with a viability >90% in a volume of 180 μl were placed into each well of a 96-well round or flat bottom tissue culture plate. All experiments were run with duplicate or triplicate cultures. In a single dose experiment, a given concentration, typically, 0.1, 1.0, or 10 μM of each drug or control was achieved at time zero and subsequently the mixtures were assayed 48 h after addition. In a multiple dose experiment, drug was added again to achieve a concentration of 0.1, 1.0, or 10 μM of each drug or control at time 0 and again after 6 and 24 h of incubation. As in single dose experiments, the cells were counted and viabilities obtained after 48 h of incubation.

After 48 h, viability was measured for the K562 cultures via trypan blue dye exclusion assay. The cells were counted on a standard hematocytometer and reported as absolute cell count.

In order to ascertain cell viability and metabolic effects of these compounds, a Sulforhodamine B (SRB) protein staining assay[20,21] was performed. This assay was performed after 48 h of incubation by fixing the cells to the 96-well plate with 50 μL of 80% TCA (for K562) and 50% TCA (for COLO 205) and incubating at 4 °C for 1 h. The plate was then washed five times with tap water and allowed to air dry. Once dry, the plate was stained by adding 0.2 mL of 0.4% SRB in 1% acetic acid to each well. The plate was again allowed to incubate at room temperature for 15−30 min at which time the plate was washed four times with 1% acetic acid and allowed to air dry. After drying, the dye was solublized by adding 0.25 mL of TRIS Base (pH 10.5). The plate was then covered with Mylar film and gently shaken to solublize all the dye. The plate was then read at 564 nm on a Hewlett-Packard 8452 spectrophometer.

Results and Discussion

The approach of preparing metal complexes of the tropolone-based structures to be used in whole cell studies is predicated upon the biological activity of tropolone and colchicine and its derivatives as antimitotics.[16,17,24] In addition, the known antineoplastic activity of Pt^{II} [1−6] and Ru^{II} complexes[7,8] make this coordination chemistry an obvious choice for exploration.

The antimitotic compounds Col and Tmca shown in Figure 1 should have an extensive coordination chemistry. As shown in Figure 2, the trimethoxybenzene ring of both could serve as an η^6 ligand to low-valent metals, the amine group of Tmca could coordinate to a variety of transition metal centers, and the tropolone unit of both could engage in bidentate coordination. In the case of Col, however, the tropolone methyl ether fragment needs to be removed by hydrolysis to take advantage of this coordination mode.

In this work we have concentrated on ways to engage the tropolone coordination mode and to test the resultant complexes as anticancer agents.

Preparation and Characterization of (bpy)Pt^{2+} and (bpy)$_2Ru^{2+}$ Derivatives. Reaction of the antimitotic ligands shown in Figure 1 with the precursor complexes (bpy)$PtCl_2$ or *cis*-(bpy)$_2RuCl_2$ was effective in favoring the tropolonato coordination mode. The choice of the (bpy)$_2Ru^{2+}$ was dictated intially by our desire to define the electronic and spectral charateristics of tropolonato donors on a probe metal fragment whose physicochemical properties are well-understood. As will be discussed later the products are suprisingly active in vitro as antineoplastics.

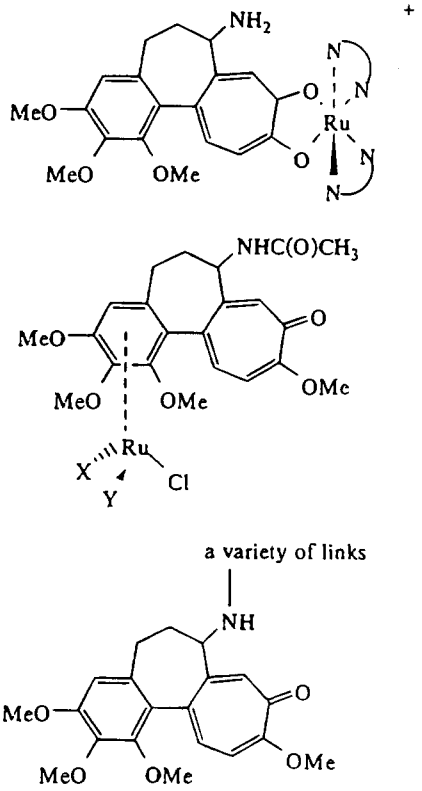

Figure 2. Possible coordination modes of the Col skeleton to transition metal complexes with illustrations drawn from the chemistry of Ru(II). Top is a tropolonato-based coordination, middle is an η^6-coordination mode, and the bottom shows likely linkages based on esters or amides.

The reaction of TpH with (bpy)$PtCl_2$ or *cis*-(bpy)$_2RuCl_2$ in water or water/ethanol followed by treatment of the solution with a PF_6^- source gave the salts, (bpy)Pt(Tp)(PF_6) or *cis*-(bpy)$_2$-Ru(Tp)(PF_6), respectively. For the platinum case, (bpy)$PtCl_2$ was prepared by a new method, that is displacement of 1,5-cyclooctadiene (COD) from (COD)$PtCl_2$ by bpy at room temperature. This route promises to be fruitful for the future preparation of bpy and phen Pt derivatives.

TmcaH yielded a similar product except that the free amine in the product was found to be protonated resulting in the formulations, (bpy)Pt($Tmca^NH$)$(PF_6)_2$ and *cis*-(bpy)$_2$Ru($Tmca^NH$)-$(PF_6)_2$ (note that the designation $Tmca^NH$ means an internal ammonium site on the ligand: see Figure 1). Elemental analysis on all the complexes were satisfactory except for (bpy)Pt-($Tmca^NH$)$(PF_6)_2$ which was found to gives variable results. This was found to be due to solvent entrainment.

For the Col reactions it was found that Pt precursors gave intractable chemistry but that a demethylation reaction occurred in the case of *cis*-(bpy)$_2RuCl_2$ to produce a complex where the tropolone methyl ether bond was demethylated and the resultant colchicine derivative (designated demethylated colchicine, DM-Col) was coordinated to Ru via the oxygens of the tropolone nucleus. The preparation is facilitated by the addition of a weak base such as acetate. During this preparation the $-NHCOCH_3$ group on the central, seven membered ring does not hydrolyze according to NMR and IR spectral data (see below). Preliminary X-ray diffraction studies of [(bpy)Pt(Tp)](PF_6) and [(bpy)$_2$-Ru(Tp)](PF_6), which will be described in an upcoming paper on Tp−metal coordination chemistry, show the planar nature of the metal−tropolone linkange.[25a]

NMR Spectral Data. ^1H NMR spectra of the complexes are complicated in the aromatic region (6−9 ppm) especially for the $Tmca^NH$ and DMCol derivatives because both the

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Table 1. Selected IR Spectroscopic Data for the Ligands and Complexes[a]

compounds	ring modes[b]	N−H or O−H modes
TpH	1608	3380 (br)
Tmca	1595	3380 (v br)
Col	1732, 1680, 1669	3313, 3237
$(bpy)_2Ru(Tp)(PF_6)$	1583	
$(bpy)_2Ru(Tmca^NH)(PF_6)_2$	1596	3259 (br)
$(bpy)_2Ru(DMCol)(PF_6)$	1673, 1590	3419
$(bpy)Pt(Tp)(PF_6)$	1612, 1592	
$(bpy)Pt(Tmca^NH)(PF_6)_2$	1607, 1594	3258 (br)

[a] All spectra as Nujol mulls. [b] These include C−O modes of the Tp ligand and aromatic C−C modes.

bipyridine and the unique ligand have many overlapping peaks as a consequence of the lack of molecular symmetry. However, by integration of the methyl and/or methoxy resonances to the aromatic region of the spectrum, the stoichiometry of the complexes was confirmed.

For the Tmca reactions, the chemical shifts for the methoxy groups do not change appreciably before and after coordination. Of note is that the peak for the amine group in free Tmca (sharp peak at 2.10 ppm in $CDCl_3$) disappears upon complexation supporting the assignment of a protonated form since exchange with trace water in the CD_3CN solvent is rapid.

In order to bolster the ^1H NMR spectral evidence and to further confirm that the $-NHCOCH_3$ group was not removed during the formation of *cis*-$(bpy)_2(RuTmca^NH)(PF_6)_2$ under basic conditions, ^{13}C NMR spectroscopy was performed. The resulting spectra show that the peaks at about 170 ppm and 22 ppm in colchicine, which can be assigned to carbonyl and methyl protons of $-NHCOCH_3$, remain after complexation.

The proton NMR spectrum of $(bpy)Pt(Tp)(PF_6)$ in CD_3CN is uncomplicated, allowing facile assignment of the resonances. The multiplet centered at 7.55 ppm and the triplet at 7.70 are seen to be coupled as are the triplets at 8.28 and 7.86. The former two are attributed to the Tp fragment. As in $TaTp^+$ the H_α and H_γ protons have merged to form the multiplet at 7.55 ppm.[25] The triplet at 7.70 ppm is therefore assigned to H_β. The remaining four protons are due to the bpy ligand. Because of the presence of Pt satellites, the doublet at 8.84 ppm is assigned to H_a. The remaining doublet must be H_d. The greater downfield shift for the para-protons vs the meta is consistent with the assigmnment of H_c for the 8.28 ppm triplet and H_b for the 7.86 ppm triplet.

Infrared Spectroscopy. The selected IR absorptions for the complexes shown in Table 1 give insight both into the course of the preparation and the nature of bonding between the drug and the metal center. The tropolone nucleus carbon−oxygen stretches are found in the range 1580−1615 cm^{-1} for both the free ligands and other troplone derivatives of Ru^{II}.[25,26] In all the complexes derived from Tmca or Col no significant changes in frequencies from the free ligands to the complexes were seen. However for the Tp complexes, a 25 cm^{-1} red shift from 1608 cm^{-1} for free ligand to 1583 cm^{-1} was found for $[(bpy)_2Ru(Tp)](PF_6)$, and from 1612 cm^{-1} to 1592 cm^{-1} for $[(bpy)Pt(Tp)](PF_6)$.

The broad region of absorption occuring at 3259 and 3258 cm^{-1} for *cis*-$(bpy)_2Ru(Tmca^NH)(PF_6)_2$ and $(bpy)Pt(Tmca^NH)(PF_6)_2$ indicates that the amine group of Tmca in the complexes are in their protonated forms since the amine group in the free ligands appear between 3400 and 3500 cm^{-1}. This is consistent with NMR spectroscopic results and elemental analysis. The amide group observed at 3419 cm^{-1} for $(bpy)_2Ru(DMCol)(PF_6)$, supports the argument that $NHCOCH_3$ group is retained after the basic reaction conditions used in the preparation (vide supra).

Table 2. Electrochemical Data for the Ruthenium Complexes[a]

complex	redox processes		
	Ru[b]	bpy1[c]	bpy2[c]
$(bpy)_2Ru(Tp)(PF_6)$	0.62	−1.47	−1.73
$(bpy)_2Ru(Tmca^NH)(PF_6)_2$	0.57	−1.48	−1.73
$(bpy)_2Ru(DMCol)(PF_6)$	0.57	−1.49	−1.73

[a] Recorded in 0.1M tetra-*N*-butylammonium hexafluorophosphate/CH_3CN medium with a Pt-button working electrode; volts vs SCE. [b] $Ru^{III/II}$ redox couple. [c] First (bpy1) and second (bpy2) 2,2′-bipyridine-based reductions.

Electrochemical Properties. Table 2 shows selected cyclic voltammetry results for only the ruthenium complexes which is provided because of the reversibility they exhibit. This feature allows for a comparison of the electronic effects by tropolone donors at a metal center since a plethora of Ru(II)−polypyridine complexes with reversible electrochemistry exist. The platinum complexes are not discussed here since their electrochemical properties are rather complicated, apparently involving chemically irreversible processes.

The redox couples in Table 2 all have cathodic to anodic peak separation of 70−80 mv, which under our conditions (glovebox electrochemical measurements), is identical to the electrochemically and chemically reversible processes in $Ru(bpy)_3^{2+}$ and *cis*-$Ru(bpy)_2Cl_2$. An indication of the ability of tropolonato-based ligands to act as a strong donors is aptly illustrated by the redox potential of the parent complex $[(bpy)_2Ru(Tp)]^+$ (0.62 V) when compared to other bis-bpy systems. For example, the $Ru^{III/II}$ potential in $Ru(bpy)_2Cl_2$ is 0.32 V (vs SCE) and that of $Ru(bpy)_2(acac)^+$ (acac is the acetylacetonate anion) is 0.57 V.[18,25] Apparently, the Tp anion is similar to acac in its donor ability. Of interest is the cathodic shift of the $Ru^{III/II}$ potential for $(bpy)_2Ru(Tmca^NH)(PF_6)_2$ and $(bpy)_2Ru(DMCol)(PF_6)$ relative to the parent (both at 0.57 vs 0.62 V). Since the overall complexes have different charges this effect must be due to an indirect electronic effect of the fused ring systems, and is just another indication that solvation energies play a small role in determining $Ru^{III/II}$ redox potentials. Figure 3 shows the cyclic voltammetry of both $(bpy)_2Ru(Tmca^NH)(PF_6)_2$ and $(bpy)_2Ru(DMCol)(PF_6)$ where the reversible nature of the $Ru^{III/II}$ couple is seen. In addition, two bpy-based reductions appear in the region of −1.5 and −1.7 V. Of particular note is the appearance of a highly irreversible process at ca. −1.0 V, which can be assigned to the reduction of the internal ammonium site to $H_2(g)$. Note that the donor nature of the tropolone nucleus is manifested by irreversible oxidation processes occurring in the range of +1.6 V for both complexes.

Another indication of the donor ability of Tp^- is provided by the the spectroelectrochemistry associated with the $Ru^{III/II}$ couple presented in Figure 4. Incremental potential sweep through the $Ru^{III/II}$ couple results in a loss of the lowest energy absorption band for $[(bpy)_2Ru^{II}(Tp)]^+$ (516 nm) and the appearance of a new band system at 732 nm. The latter can be reasonably assigned as a troplonato-to-metal charge transfer process and is a direct consequence of a low-lying Tp^--based orbital. The redox process associated with this oxidation which is seen in the cyclic voltammogram in Figure 5 at $E_p = +1.6$ V.

UV−Visible Spectroscopy. Table 3 shows the visible and ultraviolet absorption spectroscopy of the complexes recorded in CH_3CN solution. For the Ru complexes the lowest energy

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(26) (a) Griffith, W. P.; Pumphrey, C. A.; Skapski, A. C. *Polyhedron* **1987**, *6*, 891. (b) El-Hendawy, A. M.; El-Shahawi, M. S. *Polyhedron* **1989**, *8*, 2813.

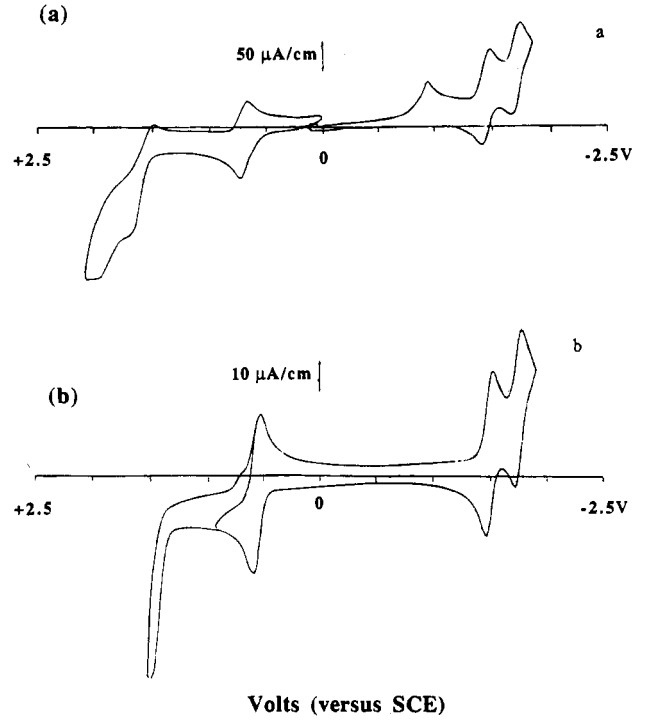

Figure 3. Cyclic voltammetry of *cis*-$(bpy)_2Ru(DMCol)(PF_6)_2$ (top; a) and *cis*-$(bpy)_2Ru(Tp)(PF_6)$ (bottom; b) in CH_3CN with 0.1 M TBAH as supporting electrolyte (Pt button working electrode and Pt wire auxillary; see text for assignments).

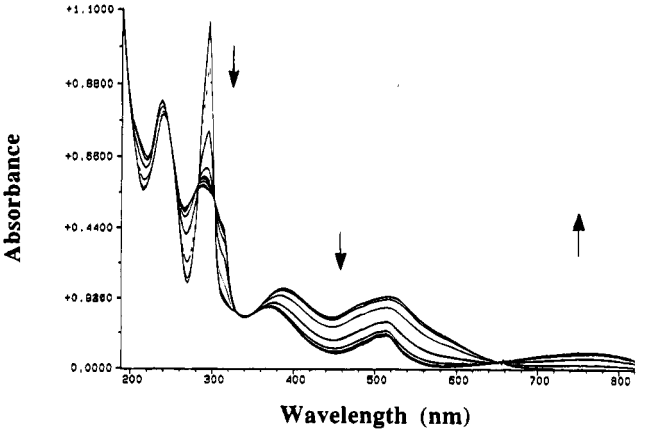

Figure 4. Spectroelectrochemistry of $(bpy)_2Ru(Tp)(PF_6)$ in CH_3CN solution at a Pt minigrid electrode. Arrows indicate the direction of spectral change as the $Ru^{III/II}$ is incrementally scanned in the anodic direction (10 mV/s) (see text).

manifold of transitions occurs at 513−516 nm and can be assigned as a metal-to-ligand charge transfer (MLCT) process by analogy to the many other similar derivatives.[18,25] The energetic position of the MLCT transitions is consistent with the tropolonato ligand exhibiting a strong donor influence. For the Pt complexes, the lowest energy transition occurs at ca. 400 nm and exhibits considerable structure, the latter is probably a consequence of overlapping MLCT and tropolonato-based transitions as has been observed for $Ta(Tp)_4^+$.[25] Three other regions of absorption are found at 340−390 nm (tropolonato-localized $\pi-\pi^*$), and at 290−320 nm and 240−250 nm (bpy-localized $\pi-\pi^*$ and phenyl-localized $\pi-\pi^*$).

Cytotoxicity Studies. The biological effects of the tropolone-based complexes were determined *in vitro* in two human cell lines, human chronic myelogenous leukemia, K562, and human colon adenocarcinoma, COLO 205. The experiments were designed to test their efficacy both in an experimental protocol

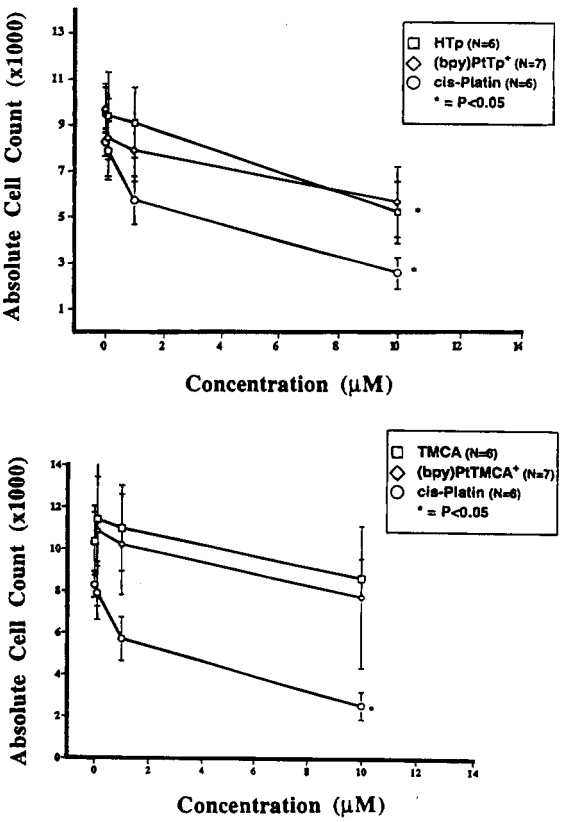

Figure 5. Single dose cytotoxicity studies of the new Pt complexes with K562 after 48 h. The top graph shows $(bpy)Pt(Tp)(PF_6)$ against TpH and cisplatin. The bottom graph shows the same experiments for $(bpy)Pt(Tmca^NH)(PF_6)_2$.

Table 3. UV−Visible Absorption Data for the Complexes in Acetonitrile

complex	λ_{max}, nm (10^{-3} ϵ, M^{-1} cm^{-1})			
$(bpy)_2Ru(Tp)(PF_6)$	516 (14.1)	387 (15.6)	294 (66.3)	238 (48.2)
$(bpy)_2Ru(Tmca^NH)(PF_6)_2$	515 (20.9)	347 (26.8)	296 (66.0)	242 (70.6)
$(bpy)_2Ru(DMCol)(PF_6)$	513 (18.3)	393 (18.3)	295 (68.9)	243 (65.7)
	345 (26.8)			
$(bpy)Pt(Tp)(PF_6)$	396 (13.0)		319 (29.3)	306 (32.5)
$(bpy)Pt(Tmca^NH)(PF_6)_2$	403 (31.7)	353 (13.5)	318 (31.7)	305 (32.0)

where a single drug dose was administered followed by an assay of cell viability, and in one where multiple drug doses were administered. The latter strategy was adopted to address the possibilty of drug degradation during cell uptake and/or metabolism or cell cycle phase specificity. Positive controls were either colchicine or cisplatin and negative controls were DMSO-RPMI 1640 media.

In single dose experiments a given concentration of compound was employed, typically 0.1, 1.0, or 10 μM at zero time followed by incubation for 48 h. After this time cytotoxicity was evaluated by trypan blue dye exclusion and/or by a SRB assay. The latter is an indirect measure of the arrest of protein synthesis and in general correlated well with the results obtained with the more classical trypan blue dye exclusion method. In multiple dosing experiments, incubation at a final drug concentration of either 0.1, 1.0, or 10 μM was performed by drug addition at 0, 6 and 24 h. The cell assays were performed at 48 h as described above.

Platinum Complex Cytotoxicity. In the K562 cell line, for single dose experiments, cisplatin was an active compound ($p < 0.0001$ at 10 μM) and Tmca and TpH were inactive ($p = 0.39$ and 0.12, respectively). Of the two new Pt complexes, $(bpy)Pt(Tp)(PF_6)$ was marginally active at 10 μM ($p = 0.055$), and $(bpy)Pt(Tmca^NH)(PF_6)_2$ was inactive at all concentrations (see Figure 5).

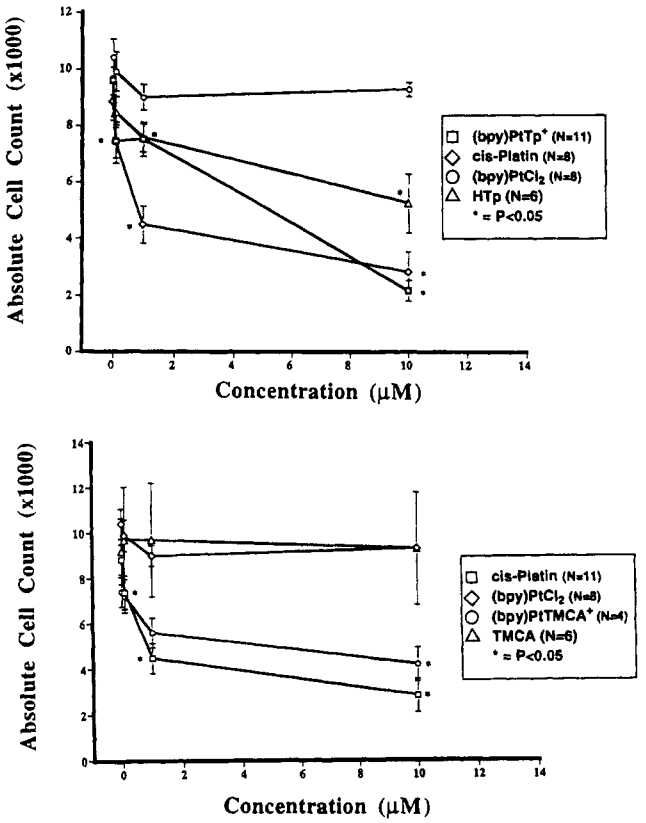

Figure 6. Multiple dose cytotoxicity studies of the new Pt complexes with K562 after 48 h. The top graph shows (bpy)Pt(Tp)(PF_6) against TpH and cisplatin and the possible saline decomposition product (bpy)-$PtCl_2$. The bottom graph shows the same experiments for (bpy)Pt-($Tmca^NH$)(PF_6)$_2$.

In the multiple dose experiments the situation was considerably different. As shown in Figure 6 both complexes had activity comparable to that of cisplatin, especially at the 10 μM level. A consistant finding was that TpH exhibited moderate activity at 10 μM. Of significance for the mechanisms involved is that the saline degradation product (bpy)$PtCl_2$ had no activity in these experiments. All the experiments shown in the figures were done using the dye exclusion cytotoxicity method, however it was found that the SRB assay method gave similar results where it was tested, i.e., in the case of cisplatin and [(bpy)Pt-(Tp)](PF_6).

Ruthenium Complex Cytotoxicity. For the Ru complexes both K562 and COLO 205 cell lines were examined for cytotoxicity, but restricting the experimental protocol to the single dose strategy only. Since the latter is an adherent cell line, the SRB assay was the method of choice. However in one set of experiments for K562, the dye exclusion method gave complementary results to that of the SRB assay for all the complexes.

The results shown below in Figure 7 for K562 incubated with (bpy)$_2$Ru(DMCol)(PF_6) are quite striking, showing cytotoxicity similar to that of colchicine itself above 0.1 μM. Figure 7 shows a comparison of (bpy)$_2$Ru(DMCol)(PF_6), [(bpy)$_2$Ru(Tp)](PF_6) and [(bpy)$_2$Ru($Tmca^NH$)](PF_6)$_2$ where it is seen that the latter two complexes also have moderate activity, but only above 10 μM. Results from the SRB assay showed that colchicine was active at 0.1 μM and above, (bpy)$_2$Ru(DMCol)(PF_6) was active at 1 μM and above, while [(bpy)$_2$Ru(Tp)](PF_6) and [(bpy)$_2$Ru-($Tmca^NH$)](PF_6)$_2$ were active only at concentrations greater than 10 μM. Suprisingly, (bpy)$_2$Ru(DMCol)(PF_6) continued to have activity near that of colchicine itself above 10 mM. The chemically robust salt [(bpy)$_3$Ru]Cl_2 was found to be inactive at all concentrations.

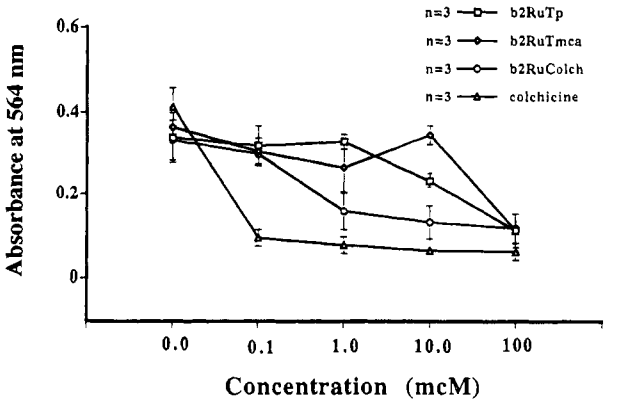

Figure 7. Single dose K562 studies for the ruthenium complexes as analyzed by the SRB assay technique (see text for details).

Because COLO 205 is an adherent cell line, all cytotoxicity experiments in COLO 205 were performed using the SRB assay. In the SRB assay, cisplatin, colchicine, [(bpy)$_2$Ru(Tp)](PF_6), and [(bpy)$_2$Ru($Tmca^NH$)](PF_6)$_2$ were weakly active at 10 μM, while (bpy)$_2$Ru(DMCol)(PF_6) was active at 1 μM and above, and [(bpy)$_3$Ru]Cl_2 was inactive at all concentrations.

Mechanistic Considerations. Several striking results are contained in the data presented above. These are (1) that the complexes [(bpy)Pt(Tp)](PF_6) and (bpy)Pt($Tmca^NH$)(PF_6)$_2$ are as active as cisplatin in multiple dose experiments involving K562, (2) that (bpy)$_2$Ru(DMCol)(PF_6) is as active as colchicine in single dose experiments with K562, and similar in activity for those involving COLO 205, and (3) that (bpy)$PtCl_2$ and [Ru-(bpy)$_3$]Cl_2 are inactive.

Given the above results, it appears that the activity is predominantly due to the *intact* complexes since solution studies of all the Ru and Pt complexes at room temperature in 0.1 M saline/5% DMSO show no reaction over 48 h as might occur due to the hydrolysis reactions depicted in eqs 1 and 2.

$$(bpy)_2Ru(DMCol)^+ + 2Cl^- \rightarrow (bpy)_2RuCl_2 + DMCol^- \quad (1)$$

$$(bpy)Pt(Tp)^+ + 2Cl^- \rightarrow (bpy)PtCl_2 + Tp^- \quad (2)$$

In these experiments, which were conducted by UV−visible spectroscopy, the spectrum of the intial complex was unchanged with no evidence for the dichloro- or mixed-aqua complexes. These observations are in accord with the general sluggish substitution kinetics for polypyridine Ru^{II} and Pt^{II} complexes.[27]

Future experiments in our laboratory will focus on the molecular mechanism of activity of this new class of tropolone-based drug. Of the several possible mechanisms, direct interaction with cellular DNA or binding to tubulin proteins are the most probable. Since previous studies have shown that bpy complexes do not exhibit appreciable DNA interaction,[28] our experimental data presented above argues in favor of a direct role for the intact metal−tropolonoid ligand complex in the observed cytotoxicity. A new mechanism for Ru- and Pt-based antineoplastic agents is likely.

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