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Shi, Yi; Toms, Bonnie B.; Dixit, Namrata; Goodisman, Jerry; and Dabrowiak, James C., "Cytotoxicity of Cu(II) and Zn(II) 2,2'-Bipyridyl Complexes: Dependence of IC 50 on Recovery Time" (2010). *Chemistry - Faculty Scholarship*. 40.

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Cytotoxicity of Cu(II) and Zn(II) 2,2'-Bipyridyl Complexes: Dependence of IC₅₀ on Recovery Time

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Received April 5, 2010

We measure the cytotoxicity of three metal complexes containing the 2,2'-bipyridine ligand, Cu(bpy)(NCS)₂, **1**, [Cu(bpy)₂(H₂O)](PF₆)₂, **2**, and Zn(bpy)₂(NCS)₂, **3**, toward neuroblastoma cells (SK-N-SH) and ovarian cancer cells (OVCAR-3) using two different cell assays. The cells were exposed to various concentrations of the compounds for 1 h and the percent inhibition of cell growth, *I*, measured for various times after exposure, i.e., as a function of the recovery time *t*. After developing the theory showing the relationship between *I* and *t*, the cytotoxicity data were analyzed to reveal that the two copper complexes, **1** and **2**, cause the cells to divide at a slower rate than the controls during the recovery period, but the zinc complex, **3**, had little or no effect on cell division during the recovery period. The usual metric for reporting cytotoxicity is IC₅₀, which is the concentration of agent required to inhibit cell growth to 50% of the control population. However, since IC₅₀ can depend on the recovery time, *t*, as is the case for **1** and **2**, reporting IC₅₀ for a single recovery time can hide important information about the long-time effects of a cytotoxic agent on the health of the cell population. Mechanistic studies with the compounds revealed that the copper complexes, **1** and **2**, cleave closed circular pBR322 DNA in the presence of ascorbate, while the zinc complex, **3**, does not facilitate DNA cleavage under the same conditions. This difference in DNA cleavage activity may be related to the fact that Cu(II) is redox active and can readily change its oxidation state, while Zn(II) is redox inert and cannot participate in a redox cycle with ascorbate to break DNA.

Introduction

Copper in its ionic forms is an important component in many metallo enzymes and proteins in the body (*1*). Since aquated copper, e.g., [Cu(H₂O)₆]²⁺, is toxic to cells, nature has developed a sophisticated mechanism for sequestering and transporting the ion from the point of its ingestion in the food supply to its ultimate incorporation in critical biological components in the cell. Imbalances in the normal levels of copper in the body lead to aberrant conditions, one of which is the neurodegenerative genetic disorder called Menkes disease. This disease, which is characterized by reduced absorption of copper, is a childhood disorder that produces delayed development, mental retardation, and early mortality (*2, 3*). In contrast, Wilson's disease, which is characterized by an excess of copper in the body, leads to reduced hepatic function and/or neurological problems that can be Parkinson-like in nature. Copper ions are also important for blood vessel development in tumors, and the ion is believed to contribute to the growth, invasion, and metastasis of cancer (*4*).

In 1965, Dwyer and co-workers reported that the Cu²⁺ complex of 3,4,7,8-tetramethyl-1,10-phenanthroline inhibits the growth of the Land-schuetz ascites tumor (*5*), and since this early report, the anticancer properties of many copper complexes containing, 1,10-phenanthroline (o-phen) and related ligands

have been actively investigated (*4, 6–9*). While the mechanism by which these complexes exert their anticancer activity is not known, it is possible that the compounds bind directly to target molecules in the cell, or since copper can readily change its oxidation state, they participate in redox chemistry that produces radicals that damage biomolecules in the cell. Recently, Dou, Verani, and their co-workers synthesized a group of Cu²⁺ complexes containing asymmetric tridentate donor ligands that induce apoptosis in C4-2B and PC-3 human prostate cancer cells (*4, 10*). Mechanistic studies with the compounds suggest that they inhibit the chymotrypsin-like activity of the 20S proteasome making them similar to the anticancer drug bortezomib, which is used for treating multiple myeloma. The Casiopeínas are a group of Cu²⁺ mixed-ligand antineoplastic agents which contain 1,10-phenanthroline or 2,2'-bipyridine (bpy) and other bidentate ligands (*11–13*). These compounds exhibit cytotoxicity, genotoxicity, and antitumor effects, but their mode of action is presently unknown.

Earlier, Koiri et al., reported that the complexes, M(bpy)-(Ac)₂H₂O, where M = Cu(II) and Zn(II), bpy = 2,2'-bipyridine and Ac = CH₃CO₂[−], bind to purified lactate dehydrogenase, LDH, and, when solutions of the compounds are perfused into mice, they are able to reduce the levels of LDH in tissues and organs (*14*). Since the inhibition of LDH blocks the interconversion of NADH and NAD⁺, a reaction that is critical for cell survival, inhibiting the enzyme with small molecules could be a useful strategy for killing cancer cells in chemotherapy (*15, 16*).

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Scheme 1. Outline of the Synthesis of the Metal Complexes Used in This Study Where LH_3^- Is the Anion of a Barbiturate

In this article, we measured the toxicity of $\text{Cu}(\text{bpy})(\text{NCS})_2$, **1**, $[\text{Cu}(\text{bpy})_2(\text{H}_2\text{O})](\text{PF}_6)_2$, **2**, and $\text{Zn}(\text{bpy})_2(\text{NCS})_2$, **3**, toward neuroblastoma (SK-N-SH) and ovarian cancer (OVCAR-3) cells. These cell lines were chosen because both neuroblastoma and ovarian cancer cells have been previously used in studies with copper and zinc complexes that are cytotoxic as well as in studies with chelators that are believed to react with cellular copper and zinc to produce compounds that are cytotoxic (17–20). By measuring the percent inhibition of cell growth, I , as a function of time after the cells have been exposed to the metal complex, i.e., as a function of the recovery time, t , it was found that surviving cells exposed to the zinc complex divide normally during the recovery period, while those cells previously exposed to the copper complexes divide more slowly during the recovery period. After deriving the relationship between I and t , we show that the measured value of IC_{50} for the two copper complexes strongly depends on t , while IC_{50} for the zinc complex is relatively independent of t . In light of earlier reports that copper–phenanthroline complexes can be activated to cleave DNA (21), we also studied the ability of the complexes to cut closed circular pBR322 DNA in the presence of the biologically common reducing agent, ascorbic acid.

Materials and Methods

Chemicals, Reagents, and Biological Supplies. 2,2'-Bipyridine, zinc(II) nitrate dihydrate, and copper(II) nitrate dihydrate were purchased from Sigma Aldrich and were used as received without further purification. All of the solvents were purchased from E. Merk and were freshly distilled prior to use. The complexes $\text{Cu}(\text{bpy})(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$ and $\text{Cu}(\text{bpy})(\text{MeCO}_2)_2 \cdot \text{H}_2\text{O}$ were synthesized using the reported procedure (22), whereas $[\text{Zn}(\text{bpy})_2(\text{H}_2\text{O})_2] \cdot (\text{LH}_3)_2 \cdot 7\text{H}_2\text{O}$ was reported by us recently (23). Elemental analysis and mass spectral measurements were carried out on a Carbo-Erba elemental analyzer 1108, and JEOL SX-102 mass spectrometer respectively. IR spectra were recorded as KBr pellets on a Varian 3100 spectrophotometer. The UV–vis measurements were made in the range 200–800 nm using a Shimadzu UV-1701 spectrophotometer. Human neuroblastoma (SK-N-SH) cells and human ovarian carcinoma (OVCAR-3) cells were purchased from the American Type Culture Collection (ATCC Number HTB-11 and HTB-161). Minimum essential medium with Earle's salts and L-glutamine

(EMEM)(10-010), RPMI 1640, fetal calf serum (FCS), cell-stripper, and trypan blue stain were from Mediatech (Herndon, VA). The cell counting kits, CCK-F and CCK-8, which determine the number of live cells in a population, were obtained from Dojindo Molecular Technologies, Inc. (Gaithersburg, MD). For the DNA cleavage studies, HEPES, agarose, Trizma base, boric acid, EDTA, and ascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO), and the plasmid DNA (pBR322), 25 $\mu\text{g}/\mu\text{L}$, was from Invitrogen (Carlsbad, CA).

Synthesis of the Complexes. The complexes **1–3** were synthesized (Scheme 1) and characterized by spectroscopic techniques as well as their single crystal X-ray crystallographic studies. The details of the X-ray studies and thermal ellipsoid diagram of complexes are given in Supporting Information.

Synthesis of $\text{Cu}(\text{bpy})(\text{NCS})_2$, **1.** A methanolic solution (10 mL) of ammonium thiocyanate (0.152 g, 2.0 mmol) was added dropwise to a methanolic solution (10 mL) of $\text{Cu}(\text{bpy})(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$ (0.361 g, 1.0 mmol). The mixture was stirred at room temperature for 2 h. A green colored precipitate was filtered out, washed with methanol, and dried *in vacuo*. The complex was crystallized in DMF and methanol. Yield: 75%. mp >530 °C. Elemental analysis calcd (%) for $\text{C}_{12}\text{H}_8\text{N}_4\text{S}_2\text{Cu}$: C, 42.85; H, 2.38; N, 16.66. Found: C, 42.98; H, 2.59; N, 16.25. IR (KBr pellet, cm^{-1}): 1604 (ν_{bpy}), 2081 (ν_{NCS}), 771 (ν_{bpy}). FAB-MS (m/z (%)): 336 [$\text{M}]^+$. UV–vis (DMSO, 10^{-4} M): $\lambda_{\text{max}}(\text{nm})$ ($\epsilon_{\text{max}} \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) 375 (3.11), 680 (0.113).

Synthesis of $[\text{Cu}(\text{bpy})_2(\text{H}_2\text{O})](\text{PF}_6)_2$, **2.** A solution of $\text{Cu}(\text{bpy})(\text{MeCO}_2)_2 \cdot \text{H}_2\text{O}$ (0.711 g, 2.0 mmol) in DMF (10 mL) was refluxed for 5 h on a sand bath followed by the addition of excess of sodium acetate while stirring. After 5 h, the solution was filtered, and an aqueous solution of NH_4PF_6 was added to the filtrate. A solid crystalline product (light blue) was obtained, and single crystals suitable for X-ray diffraction study were picked up. Yield: 61%. mp >230 °C. Elemental analysis calcd (%) for $\text{C}_{20}\text{H}_{18}\text{CuF}_{12}\text{N}_4\text{OP}_2$: C, 35.13; H, 2.65; N, 8.19. Found: C, 35.18; H, 2.64; N, 8.12. IR (KBr pellet, cm^{-1}): 1601 (ν_{bpy}), 846 (ν_{PF_6}), 771 (ν_{bpy}). FAB-MS (m/z (%)): 682(48) [$\text{M}]^+$. UV–vis (DMSO, 10^{-4} M): $\lambda_{\text{max}}(\text{nm})$ ($\epsilon_{\text{max}} \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) 311 (3.86), 349sh (0.48), 691 (0.213).

Synthesis of $\text{Zn}(\text{bpy})_2(\text{NCS})_2$, **3.** A solution of supramolecular complex $[\text{Zn}(\text{bpy})_2(\text{H}_2\text{O})_2](\text{LH}_3)_2 \cdot 7\text{H}_2\text{O}$ (1.208 g, 1.0 mmol) in DMSO (10 mL) was mixed with a solution of ammonium thiocyanate (0.152 g, 2.0 mmol) in methanol (10 mL), and the reaction was carried out by stirring at room temperature for 2 h.

The reaction mixture was left for slow evaporation at room temperature. Block shaped colorless crystals were obtained after a week. Elemental analysis calcd (%) for C₂₂H₁₆N₄S₂Zn: C, 53.44; H, 3.24; N, 11.33. Found: C, 53.88; H, 3.39; N, 11.23. IR (KBr pellet, cm⁻¹): 1595 (ν_{bpy}), 2070 (ν_{NCS}), 771 (ν_{bpy}). FAB-MS (m/z (%)): 495 [M]⁺. UV-vis (DMSO, 10⁻⁴ M): $\lambda_{\text{max}}(\text{nm})$ ($\epsilon_{\text{max}} \times 10^4$ M⁻¹ cm⁻¹) 280 (3.37).

Cytotoxicity Studies. The studies involving human neuroblastoma (SK-N-SH) cells and human ovarian carcinoma (OVCAR-3) cells were carried out under standard conditions in a humidified, 37 °C, 5% CO₂ atmosphere in an incubator. The culture medium used for SK-N-SH cells was Eagle's minimum essential medium (EMEM) to which had been added 10% fetal calf serum (FCS), 100 $\mu\text{g/mL}$ streptomycin, 100 IU/mL penicillin, and 2.0 mM L-glutamine. The culture medium used for OVCAR-3 was RPMI 1640 (Roswell Park Memorial Institute) to which had been added 20% fetal calf serum (FCS), 100 $\mu\text{g/mL}$ streptomycin, 100 IU/mL penicillin, 2.0 mM L-glutamine, and 10 $\mu\text{g/mL}$ insulin. Four solutions of the complex in the medium having concentrations 250, 500, 1000, and 2000 μM were prepared by dissolving a weighed amount of complex in 0.3 mL of DMSO followed by the addition 5.7 mL of culture medium to give the indicated final compound and DMSO (5%) concentrations. To each of the 60 wells, 6 groups of 10 wells each, in the central portions of four 96-well microplates were added, medium alone containing no cells, Group 1, and medium containing 5×10^4 cells/mL, Groups 2–6. The cells were allowed to grow for 24 h reaching ~30% confluency after which time the medium was removed and replaced with 100 μL of fresh medium containing 5% DMSO, Group 2, or 100 μL of medium containing compound having concentrations of 250, 500, 1000, and 2000 μM for Groups 3–6, respectively. After an exposure time to the copper/zinc compound of 1 h, the medium in the wells containing cells was removed and replaced with fresh medium, and the cells were allowed to recover for 2, 26, 50, and 74 h at the end of which time the number of live cells was determined using the CCK-F assay according to the specified protocol. The CCK-F assay relies on esterases in live cells to convert a precursor compound into a calcein dye, the amount of which can be quantitated by fluorescence. The viability of SK-N-SH cells was also determined using a second method, the CCK-8 assay, following the same procedure. The CCK-8 assay relies on dehydrogenases in live cells to convert a tetrazolium salt into formazan dye, the amount of which can be quantitated by absorption spectroscopy. The concentrations of the copper/zinc complex used were 25 μM , 250 μM , and 2500 μM .

The percent inhibition of **1–3** was calculated using eq 1, where I is the percent inhibition, A_{D} is the absorbance or fluorescence of wells containing cells + culture medium + 5% DMSO, A_{T} is the absorbance or fluorescence of wells with cells containing culture medium + 5% DMSO + the copper/zinc complex, A_{C} is the absorbance or fluorescence of wells with cells and culture medium, and A_{M} is the absorbance or fluorescence of wells without cells but with culture medium.

$$I = 100 \times \left[\frac{A_{\text{D}} - A_{\text{T}}}{A_{\text{C}} - A_{\text{M}}} \right] \quad (1)$$

DNA Cleavage Studies by Agarose Gel Electrophoresis. Six solutions, 20 μL each, in 10 mM HEPES buffer (pH 7.4), each containing various final concentrations of **1** (1, 2, 4, 6, 8, and 10 μM), **2**, (10, 20, 40, 60, 80, and 100 μM), and **3** (10, 20, 30, 40, 50, and 60 μM) and plasmid DNA (33.3 μM base pairs) were prepared. Since stock solutions of the complex were prepared in a manner analogous to those described for the cell studies, the reaction solutions also contained 5% DMSO. The reaction solutions, along with solutions containing DNA alone in 10 mM HEPES buffer with and without 5% DMSO, were incubated at 37 °C for 30 min. After this time, a stock solution of ascorbic acid (final concentration, 1.0 mM) was added to the complex containing the solutions, and the resulting mixtures were

incubated at 37 °C for an additional 30 min to produce DNA strand cleavage. As a control, DNA in buffer and 5% DMSO was incubated with complexes **1** (5 and 10 μM), **2**, and **3** (50 and 100 μM) for 0.5 h in the absence of ascorbic acid. Following incubation, the reaction mixtures were quenched by rapid cooling using dry ice, and 2.5 μL of a loading buffer containing 50% glycerol, 0.25% bromophenol blue, and 0.25% xylene cyanol was added to each sample. An 8 μL volume of each solution was loaded onto a 1% (w/v) agarose gel in 0.5 $\times$ (0.045 M Tris-borate and 0.001 M EDTA) TBE buffer (pH 8.0), and electrophoresis was carried out at 100 V for 4 h. The gel was exposed to a 5×10^{-5} % solution of ethidium bromide for 30 min and washed (destained) in distilled water for 15 min. An image of the gel was captured using a Kodak Gel Logic 100 imaging system.

Results

Figures 1 and 2 show the percent inhibition, I , of SK-N-SH neuroblastoma cells (Figure 1) and OVCAR-3 ovarian cancer cells (Figure 2) by Cu(bpy)(NCS)₂, **1**, [Cu(bpy)₂(H₂O)](PF₆)₂, **2**, and Zn(bpy)₂(NCS)₂, **3**, as functions of concentration for various recovery times. The curves shown in Figures 1 and 2 are the best fits to the function

$$I = 100(1 - e^{-\alpha c}) \quad (2)$$

where I is the percent inhibition of cell growth, α is the molar inhibition parameter, and c is the concentration of the metal complex. The measured percent inhibition I is equal to

$$I = 100(1 - N_{\text{t}}/N_{\text{u}}) \quad (3)$$

where N_{t} is the number of live cells in a well treated with metal complex and N_{u} the number of live cells in a well not treated with **1–3**; $N_{\text{t}}/N_{\text{u}}$ is obtained from the absorbance or fluorescence according to eq 1.

We suppose that the cells reproduce with growth constant k ; therefore, if the number of live cells at time 0 is N_0 , the number of live cells at time t is $N_0 e^{kt}$. If the only effect of the metal complex is to kill a fraction of the cells, f , without affecting the health of the surviving cells, the number of live cells at time 0 is $(1 - f)N_0$, and the number of live cells at time t is $(1 - f)N_0 e^{kt}$. It is easy to see from eq 3 that I will be independent of the recovery time t and always equal to $100f$.

It is expected that f depends on the concentration of drug used, c , becoming zero for $c = 0$ and approaching 1 for $c \rightarrow \infty$. A simple representation of this is: $f = 1 - \exp(-\alpha c)$, as used in eq 2. For small c , f is proportional to c ; the initial slope of a plot of I vs c is 100α . The IC₅₀ is simply equal to $\ln(2)/\alpha$. This gives a single number for IC₅₀ only in the simplest case, when I depends on c but not on recovery time t . It is clear from the calculated IC₅₀ values, given in Table 1, as well as from the initial slopes in Figures 1 and 2, that this is not at all the case for the copper complexes considered here.

The initial slopes of the plots almost always increase with the recovery time. The reason is that the copper complexes, in addition to killing a fraction f of the cells during the time that the cells are exposed to the agent, have a long-lasting effect on the health of the surviving cells. They continue to hinder the division of the surviving cells after the agent is removed, i.e., during the recovery period. Thus, simply measuring the IC₅₀ by measuring f at one recovery time may hide important information about this additional effect.

If the effects of the drug are long-lasting, so that the surviving cells are in less than perfect health, then they will have a decreased growth rate k' with $k' < k$. Then, assuming a fraction

Figure 1. Percent inhibition of cell growth vs compound concentration on SK-N-SH cells. The cells were exposed to a compound for 1 h and incubated on separate plates for 2, 26, 50, and 74 h before measurement. Data collected from CCK-8 and CCK-F assays were represented by squares and diamonds, respectively. The curves were the best fits of eq 1 to the data from CCK-F assays. The first four panels, results for $\text{Cu}(\text{bpy})(\text{NCS})_2$, **1**; next four panels, results for $[\text{Cu}(\text{bpy})_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$, **2**; last four panels, results for $\text{Zn}(\text{bpy})_2(\text{NCS})_2$, **3**.

f of the original cells were killed, the number of treated cells at time t is

$$N_t(t) = (1 - f)N_0e^{k't}$$

and the percent inhibition is

$$I = 100 \frac{N_0e^{kt} - (1 - f)N_0e^{k't}}{N_0e^{kt}} = 100[1 - (1 - f)e^{(k' - k)t}] \quad (4)$$

Since $\exp[(k' - k)t]$ in the last member is less than one, the percent inhibition, I , will increase with t . For a fixed value of t , let us abbreviate $e^{(k' - k)t}$ by $C(t)$. Then, inserting the concentration dependence of f , $f = 1 - \exp(-\alpha c)$, we have

$$I = 100[1 - C(t)e^{-\alpha c}] \quad (5)$$

It may be expected that $C(t)$ depends on concentration c as well as on t . However, the small number of data points in the case of the metal complexes in this article (3 to 5 in each case) and the relatively large experimental errors do

not justify using a two-parameter function to fit the data. Thus we have used eq 2 instead of eq 5 in Figures 1 and 2, corresponding to $C = 1$. If, however, $C(t)$ really decreases with t , the second term in eq 5 will be smaller for larger t values, which will appear as an increase in the value of α (molar inhibition parameter) with t .

It is apparent from the figures that the fits to eq 2 are good overall. This is shown by the values of r^2 (goodness of fit), which are calculated according to

$$r^2 = 1 - \frac{\sum_j (I_j - \bar{I}^{\text{cal}})^2}{\sum_k (I_j - \bar{I})^2} \quad (6)$$

Here I_j is the measured inhibition for the j^{th} complex concentration, $\bar{I}$ is the mean value of I_j , and $\bar{I}^{\text{cal}}$ the corresponding

Figure 2. Percent inhibition of cell growth vs compound concentration on OVCAR-3 cells. The cells were exposed to a compound for 1 h and incubated on separate plates for 2 h, 26 h, 50 h, and 74 h before measurement. Data collected from CCK-F assays was represented by diamonds. The curves were the best fits of eq 1 to the data from CCK-F assays. The first four panels, results for $\text{Cu}(\text{bpy})(\text{NCS})_2$, **1**; next four panels, results for $[\text{Cu}(\text{bpy})(\text{H}_2\text{O})_2](\text{PF}_6)_2$, **2**; last four panels, results for $\text{Zn}(\text{bpy})_2(\text{NCS})_2$, **3**.

Table 1. Molar Inhibition Parameter, α , r^2 and IC_{50} Values for All Complexes and Recovery Times, Derived from the Results Plotted in Figures 1 and 2^a

SK-N-SH cells				OVCAR-3 cells			
complex, recovery time	α (μM^{-1})	r^2	$IC_{50}(\mu M)$	complex, recovery time	α (μM^{-1})	r^2	$IC_{50}(\mu M)$
1 , 2 h	0.000148	0.699	4683	1 , 2 h	0.000120	0.628	5776
1 , 26 h	0.000190	0.490	3648	1 , 26 h	0.000914	0.857	758
1 , 50 h	0.000266	0.895	2606	1 , 50 h	0.001227	0.880	565
1 , 74 h	0.000645	0.956	1075	1 , 74 h	0.004606	0.712	150
2 , 2 h	0.000700	0.594	990	2 , 2 h	0.000117	0.265	5924
2 , 26 h	0.001419	0.723	488	2 , 26 h	0.000803	0.924	863
2 , 50 h	0.001728	0.745	401	2 , 50 h	0.002144	0.940	323
2 , 74 h	0.001840	0.782	377	2 , 74 h	0.001221	0.679	568
3 , 2 h	0.000493	0.978	1406	3 , 2 h	0.000534	0.936	1298
3 , 26 h	0.001238	0.865	560	3 , 26 h	0.000676	0.862	1025
3 , 50 h	0.001010	0.822	686	3 , 50 h	0.000490	0.830	1415
3 , 74 h	0.000873	0.818	794	3 , 74 h	0.000463	0.798	1497

^a Cu(bpy)(NCS)₂, **1**, [Cu(bpy)₂(H₂O)](PF₆)₂, **2**, and Zn(bpy)₂(NCS)₂, **3**.

inhibition calculated according to eq 2. Table 1 shows the r^2 values for all 24 conditions employed in this study, as well as the molar inhibition parameter α for each.

As shown above, one can determine whether there are long-lasting, effects on the cells (in addition to the initial cell kill produced by exposure to the agent) by considering how the molar inhibition parameter α depends on recovery time t . If, for example, the agent hinders cell division but the cells remain competent to register as being alive with a standard cell assay, α will increase with t . If there are no effects of the agent on the cells after the initial kill, α will be constant or independent of t during the recovery period.

In order to determine whether α depends on t , we have performed linear fits on all six data sets given in Table 1 (of course, it is not expected that α is a linear function of t ; the linear fits are simply to establish slopes). The following results were obtained. For **1**, the slope is $(7.8 \pm 3.1) \times 10^{-6} \mu M^{-1} h^{-1}$ for SK-N-SH and $(5.7 \pm 2.0) \times 10^{-5} \mu M^{-1} h^{-1}$ for OVCAR-3, i.e., the slope is definitely positive. This indicates that, in addition to killing some cells during the 1 h exposure time, compound **1** produces long-term effects, in that cells divide more slowly during the recovery period. For **2**, the slope is $(1.6 \pm 0.4) \times 10^{-6} \mu M^{-1} h^{-1}$ for SK-N-SH and $(1.9 \pm 1.4) \times 10^{-5} \mu M^{-1} h^{-1}$ for OVCAR-3. Since these slopes are definitely positive, **2** also slows cell division during the recovery period. For **3**, the slope is $(3.8 \pm 6.6) \times 10^{-6} \mu M^{-1} h^{-1}$ for SK-N-SH and $(-1.7 \pm 1.8) \times 10^{-6} \mu M^{-1} h^{-1}$ for OVCAR-3, i.e., the slopes are essentially zero in both cases. Thus, the zinc complex differs from the copper complexes in that the latter complex hinders cell division during recovery, but the zinc complex, which, like the copper complexes, kills cells during the exposure period, does not affect cell division during the recovery period.

It is implied that IC_{50} may depend on recovery time if the agent being tested affects the division rate of cells after exposure to the agent. In Figures 3 and 4 and Table 2, we consider how the ratio of treated to nontreated cells, N_t/N_u , depends on t for a fixed concentration of the complex. If there are no effects on division, N_t/N_u will be independent of t , but if division is hindered due to exposure to the agent, N_t/N_u will decrease with t . It may also happen that N_t/N_u increases with t , which means that the surviving cells divide more rapidly than the untreated cells after exposure to the agent. Table 2 shows the results of linear fits of N_t/N_u vs recovery time for a fixed concentration of the metal complex. For the copper compounds, the slope is definitely negative (magnitude of the slope greater than the standard error) for both assays and both cell types except for one case. For SK-N-SH cells with the CCK-8 assay and 25 μM **1**, the slope is negative, but its magnitude is less than the

standard error, indicating that the slope is statistically zero. It is clear that the two copper complexes diminish the reproductive capacity of the cells which survive the initial exposure to the complex.

For the zinc compound, **3**, the situation is quite different in that the slope is statistically zero in three cases, statistically negative in only one case, and statistically positive in five cases. This shows that the reproductive capacity of the surviving cells which have been exposed to the zinc complex is certainly not diminished; in fact, the metal complex may even enhance division.

Finally, we compare the results of the two assays, CCK-8 and CCK-F, for neuroblastoma, SK-N-SH cells (Figure 1). In the CCK-8 assay, a tetrazolinum salt is taken up by a live cell and reduced by dehydrogenases to a formazan dye, the concentration of which is measured by absorption spectroscopy. In the CCK-F assay, a fluorescein precursor is taken up by a live cell and hydrolyzed by esterases to a fluorescein dye, which is detected by fluorescence. As is evident in Figure 1, in 32 out of 36 points, the measured inhibition for the CCK-8 assay is within one standard deviation of the curve obtained by fitting the inhibitions measured using the CCK-F assay. The agreement suggests that both assays are detecting the same number of live cells.

Since copper-*o*-phenanthroline complexes are known to cleave DNA in the presence of activating agents (21), the ability of the 2,2'-bipyridine compounds **1**–**3** to cleave the closed circular pBR322 DNA in the presence and absence of ascorbate was also studied. As is evident from Figure 5, **1** and **2** can be activated by ascorbate to cut closed circular DNA, eventually reducing the plasmid to low molecular weight fragments, which appear as an ethidium bromide-stained streak in the gel. This behavior contrasts with that of Zn(bpy)₂(NCS)₂, **3**, Figure 5c, which produces no DNA cleavage in the presence of ascorbate. In the absence of ascorbic acid, none of the complexes produced DNA cleavage (Figure 5d).

Discussion

In this work, we report the synthesis, characterization, and cytotoxic properties of Cu(bpy)(NCS)₂, **1**, [Cu(bpy)₂(H₂O)](PF₆)₂, **2**, and Zn(bpy)₂(NCS)₂, **3**. Structurally, **1** is a 4-coordinate approximately square planar Cu(II) complex with N-bonded thiocyanate ligands, **2** is a 5-coordinate, trigonal bipyramidal Cu(II) complex with a coordinated water molecule, and **3** is a 6-coordinate approximately octahedral Zn(II) compound with two N-bonded thiocyanate ligands (Scheme 1 and Supporting Information). The cytotoxicities of the metal

Figure 3. Ratio N_t/N_u is plotted against recovery time t for a fixed concentration of agent, where N_t = number of cells in treated population, and N_u = number of cells in untreated population. N_t and N_u were measured using the CCK-F assay. The first six plots are for compound **1**, the next six for compound **2**, and the last six for compound **3**. For the copper compounds **1** and **2**, N_t/N_u decreases with t , but this is not the case for the zinc compound **3**.

complexes toward neuroblastoma, SK-N-SH, and ovarian cancer, OVCAR-3, were measured using two standard assays that detect the number of live cells in a population. Cells were exposed to the compound for 1 h. In order to uncover long lasting effects that the complexes may have on the cells after exposure, the number of living cells was determined for different recovery times. The results reveal that the copper complexes, **1** and **2**, impair the ability of the cells to divide, whereas the zinc complex, **3**, has no, or possibly a weak stimulating, effect on cell division during the recovery period (Figures 1 and 2 and

Table 1). We point out that, since IC_{50} , the concentration of agent required to inhibit cell growth to 50% of the control population, is equal to $\ln(2)/\alpha$, and α , the molar inhibition parameter, can depend on recovery time, reporting IC_{50} for a single recovery time can hide important information concerning the health of the population after exposure to the toxin.

In this case, the hidden information is that while the copper complexes, **1** and **2**, kill some of the SK-N-SH and OVCAR-3 cells during the 1 h exposure period, the compounds also have a longer-lasting effect on the surviving cells, causing them to

Figure 4. Ratio N_t/N_u is plotted against recovery time t for a fixed concentration of agent, where N_t = number of cells in treated population, and N_u = number of cells in untreated population. N_t and N_u were measured using the CCK-8 assay. The first three plots are for complex **1**, the next three for complex **2**, and the last three for complex **3**. For the copper compounds **1** and **2**, N_t/N_u decreases with t , but this is not the case for the zinc compound **3**.

Table 2. Slopes with Errors of Plots of N_t/N_u vs Time for a Fixed Concentration of Complex^a

compound	cells	assay	concn (μ M)	slope (h^{-1})	error (h^{-1})	sign of slope
1	SK-N-SH	CCK-F	500	-0.00824	0.00292	-
			1000	-0.00458	0.00199	-
			2000	-0.00114	0.00020	-
		CCK-8	25	-0.00217	0.00348	0
			250	-0.00722	0.00366	-
	OVCAR-3	CCK-F	2500	-0.00623	0.00203	-
			500	-0.00884	0.00411	-
			1000	-0.01336	0.00542	-
			2000	-0.00535	0.00471	-
			2000	-0.00460	0.00135	-
2	SK-N-SH	CCK-F	500	-0.01281	0.00173	-
			1000	-0.00676	0.00232	-
			2000	-0.00460	0.00135	-
		CCK-8	25	-0.00194	0.00019	-
			250	-0.00401	0.00089	-
	OVCAR-3	CCK-F	2500	-0.00717	0.00234	-
			500	-0.01281	0.00173	-
			1000	-0.01192	0.00135	-
			2000	-0.00885	0.00453	-
			2000	-0.00415	0.00315	-
3	SK-N-SH	CCK-F	500	0.00091	0.00201	0
			000	-0.00236	0.00318	0
			2000	-0.00415	0.00315	-
		CCK-8	25	0.00336	0.00023	+
			250	0.00351	0.00073	+
	OVCAR-3	CCK-F	2500	0.00375	0.00238	+
			500	0.00119	0.00065	+
			1000	0.00369	0.00088	+
			2000	-0.00119	0.00192	0
			2000	-0.00119	0.00192	0

^a Cu(bpy)(NCS)₂, **1**, [Cu(bpy)₂(H₂O)](PF₆)₂, **2**, and Zn(bpy)₂(NCS)₂, **3**.

divide more slowly than cells of a control group. This is shown in the slopes of plots of N_t/N_u vs recovery time t (Figures 3 and 4 and Table 2). The longer the value of t , the greater the apparent

inhibition (proportional to $1 - N_t/N_u$) for a given concentration of compound. This means longer t lowers the apparent value of IC_{50} , i.e., the compounds appear more cytotoxic for larger t . As shown in Table 1, the IC_{50} of the two copper complexes **1** and **2** is diminished by more than an order of magnitude when the recovery time is increased from 2 to 74 h for both cell lines studied. Structurally, **1** contains one 2,2'-bipyridine ligand, and the compound is uncharged, while **2** has two 2,2'-bipyridine ligands, and the complex is a divalent cation (Scheme). Interestingly, **2** is clearly more toxic than **1** toward neuroblastoma cells, but there is no apparent difference in the toxicity of the compounds toward the ovarian cancer cell line (Table 1). Determining if this difference in cytotoxicity toward the two cell lines is related to the charge on the compounds and the mechanism by which they are transported into the cell and/or the number of attached 2,2'-bipyridine ligands attached to the metal ion will require additional study.

As is evident from Table 1, the zinc complex **3** does not show a decrease in IC_{50} with t , as do the copper complexes. Correspondingly, the slopes in Table 2, representing the time derivatives of N_t/N_u , are definitely negative for all concentrations of both copper complexes but probably positive for most of the zinc complex results. Thus, it may be that **3** enhances the ability of cells that survive the initial attack to divide or that the cells which survive the attack by **3** are also those which inherently divide faster than the others in the population. This would be an example of drug-induced resistance, since cells which survive treatment are healthier than before. It is interesting that the copper complexes do not have the same effect.

We are unaware of any published study which analyzes, as described herein, the dependence of inhibition on recovery time,

Figure 5. Agarose gel electrophoresis images of **1** (a), **2** (b), and **3** (c) cleavage of pBR322 DNA in the presence of the activator, ascorbic acid (1 mM, 0.5 h), and a control group (d) showing the effects of the metal complexes alone on DNA in the absence of the activator. The studies were done in 1 mM HEPES buffer with 5% (v/v) DMSO, pH 7.4, at 37 °C. In panels a–c, lane 1, DNA alone; lane 2, DNA plus 5% DMSO; lane 3, DNA with ascorbic acid, no complex. (a) For lanes 4–6, which contained ascorbic acid, the lane numbers and [1]/[DNA-base pairs] are as follows: 4, 0.03; 5, 0.06; 6, 0.12; 7, 0.18; 8, 0.24; 9, 0.30. (b) For lanes 4–6, which contained ascorbic acid, the lane numbers and [2]/[DNA-base pairs] are as follows: 4, 0.3; 5, 0.6; 6, 1.2; 7, 1.8; 8, 2.4; 9, 3.0. (c) For lanes 4–6, which contained ascorbic acid, the lane numbers and [3]/[DNA-base pairs] are as follows: 4, 0.3; 5, 0.6; 6, 0.9; 7, 1.2; 8, 1.5; 9, 1.8. A control group without ascorbic acid is shown in panel d. Lane 1, DNA alone; lane 2, DNA plus 5% DMSO. For lanes 3 and 4, [1]/[DNA-base pairs] are 0.15 and 0.30; for lanes 5 and 6, [2]/[DNA-base pairs] are 0.3 and 3.0; for lanes 7 and 8, [3]/[DNA-base pairs] are 0.3 and 3.0. After electrophoresis, the DNA was stained with ethidium bromide.

in order to obtain information about long-time effects of a cytotoxic agent on cells which survive treatment. However, recovery time was noted to be important in Murine leukemia L1210 cells treated with cisplatin, in that low concentrations of the drug caused the cells to be transiently arrested in the G₂ phase for about 3 days before this phase is bypassed, and growth is resumed (24). In another study, cisplatin analogues containing sulfur carrier ligands affected the growth of MCF7 human breast cancer cells during the recovery period (25). Interestingly, in this study both inhibitory and stimulatory effects on cell division were observed, depending on the platinum complex being investigated. This makes the reported value of IC₅₀ strongly dependent on the time (recovery period) at which inhibition is measured.

Obviously, the amount of inhibition observed for a cell population depends not only on the concentration of cytotoxic agent but also on the length of time that the cells are in contact with the agent, the exposure time. It is believed that the relevant parameter is the AUC, the area under the curve, giving drug concentration vs time (2). If this curve resembles a rectangle, then $AUC = \text{concentration} \times \text{exposure time}$. In an earlier study by us involving Jurkat cells and cisplatin (26), the percent viability of the cell population measured 24 h after exposure to the drug correlated inversely with AUC, where AUC was calculated as the product of exposure time and drug concentration. If the amount of cell death in fact depends only on AUC, one can obtain the same information about inhibition using any convenient exposure time. We have used 1 h in the experiments presented here. In a very crude way, this approximates a short i.v. administration of cisplatin and its rapid clearance from blood, $t_{1/2} \sim 0.5$ h (2).

While this study provides no detailed mechanistic information on how the 2,2'-bipyridine complexes are able to kill neuroblastoma and ovarian cancer cells, the fact that all of the complexes have the bpy ligand and only the copper complexes are cytotoxic suggests that the coordinated copper ion is important in slowing the division of and killing cells. Since it is known that Cu(II) in the biological system can facilitate the production of the hydroxyl radical, hydrogen peroxide, and

superoxide, collectively referred to as ROS (2, 21, 27), it is possible that **1** and **2** induce ROS which damage biological molecules in the cell. This mechanism, which may be operating in observed radical damage to biological molecules in Alzheimer's disease, requires a reducing agent, molecular oxygen, and a change in oxidation state of the copper ion (2). Since DNA is an important target for anticancer agents, we investigated the ability of **1–3** to cleave closed circular pBR322 DNA. As is evident in Figure 5a and b, the two copper compounds, **1** and **2**, facilitate breakage of closed circular pBR322 DNA in the presence of an excess of the common biological reducing agent ascorbic acid in a manner which is dependent on the concentration of the complex present in the medium. Since molecular oxygen was not excluded in the reaction, this observation is consistent with copper mediated damage to DNA through the production of ROS (2). Figure 5c also shows that the zinc complex, **3**, cannot break DNA under the same activating conditions. Since Zn(II) cannot easily change its oxidation state, this observation is also consistent with **1** and **2** damaging DNA via a ROS generating type mechanism. In the absence of ascorbic acid, none of the complexes cut pBR322 DNA, Figure 5d, which shows the importance of the reducing agent in the cleavage process.

Earlier, it was suggested that lactate dehydrogenase, LDH, may be a biological target for $M(\text{bpy})(\text{Ac})_2 \cdot \text{H}_2\text{O}$, where $M = \text{Cu(II)}$ and Zn(II) , bpy = 2,2'-bipyridine, and $\text{Ac} = \text{CH}_3\text{CO}_2^-$, compounds which are similar in structure to **1–3** (14). The CCK-8 cell assay used in this investigation requires viable cellular dehydrogenases for reducing a tetrazolium salt to a formazan dye, while the CCK-F assay requires viable esterases for producing a detectable fluorophore for quantitating the number of live cells. In view of the fact that both assays, within error, yield the same number of live cells, it would seem that the killing mechanism used by these compounds does not involve either esterases or dehydrogenases. Clearly, additional work will be needed to uncover the molecular mechanism by which these interesting compounds kill cancer cells.

Conclusions

The cytotoxicities of three compounds, Cu(bpy)(NCS)₂, **1**, [Cu(bpy)₂(H₂O)](PF₆)₂, **2**, and Zn(bpy)₂(NCS)₂, **3**, toward neuroblastoma, SK-N-SH, and ovarian cancer, OVCAR-3, cells were measured. Cells were exposed to a metal complex for 1 h, after which the agent was removed and the number of live cells measured using two standard cell counting assays. For the two copper complexes, the results depend strongly and interestingly on the time allowed to elapse between the removal of the metal complex and the measurement of the number of live cells in the population, i.e., the recovery time *t*, showing that the measured value of IC₅₀ strongly depends on the recovery time. We suggest that when measuring the cytotoxicity of an agent in a cell study it is important to measure the percent inhibition and IC₅₀ at various recovery times, which will uncover the long-term effects of the toxin on the health of the cell population. In contrast to the copper complexes, the zinc complex, **3**, was only weakly cytotoxic and had little or no effect on cell division during the recovery period.

This study also showed that in the presence of ascorbate, Cu(bpy)(NCS)₂, **1**, and [Cu(bpy)₂(H₂O)](PF₆)₂, **2**, cleave closed circular pBR322 DNA, while Zn(bpy)₂(NCS)₂, **3**, cannot induce DNA strand scission, an observation which is consistent with the redox inertness of the Zn(II) ion in **3**. Additional work will be needed to determine if DNA and/or proteins are targets for these metal complexes in the cell.

Supporting Information Available: X-ray crystallographic data [deposited in the Cambridge Crystallographic Data Centre as CCDC 771189 (complex **1**), 647959 (complex **2**) and 647855 (complex **3**)] collection details, figures showing thermal ellipsoids, and a table for crystallographic data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Roat-Malone, R. M. (2002) *Bioinorganic Chemistry. A Short Course*, John Wiley & Sons, Inc., Hoboken, NJ.
- (2) Dabrowiak, J. C. (2009) *Metals in Medicine*, Wiley, Chichester, UK.
- (3) Wang, T., and Guo, Z. (2006) Copper in medicine: homeostasis, chelation therapy and antitumor drug design. *Curr. Med. Chem.* **13**, 525–537.
- (4) Chen, D., Milacic, V., Frezza, M., and Dou, Q. P. (2009) Metal complexes, their cellular targets and potential for cancer therapy. *Curr. Pharm. Des.* **15**, 777–791.
- (5) Dwyer, F. P., Mayhew, E., Roe, E. M., and Shulman, A. (1965) Inhibition of landschuetz ascites tumour growth by metal chelates derived from 3,4,7,8-tetramethyl-1,10-phenanthroline. *Br. J. Cancer.* **19**, 195–199.
- (6) Tardito, S., and Marchiò, L. (2009) Copper compounds in anticancer strategies. *Curr. Med. Chem.* **16**, 1325–1348.
- (7) Maheswari, P. U., van der Ster, M., Smulders, S., Barends, S., van Wezel, G. P., Massera, C., Roy, S., den Dulk, H., Gamez, P., and Reedijk, J. (2008) Structure, cytotoxicity, and DNA-cleavage properties of the complex [Cu(II)(pbt)Br₂]. *Inorg. Chem.* **47**, 3719–3727.
- (8) Thati, B., Noble, A., Creaven, B. S., Walsh, M., Kavanagh, K., and Egan, D. A. (2007) Apoptotic cell death: a possible key event in mediating the in vitro anti-proliferative effect of a novel copper(II) complex, [Cu(4-Mecdoa)(phen)(2)] (phen=phenanthroline, 4-Mecdoa=4-methylcoumarin-6,7-dioxacetate), in human malignant cancer cells. *Eur. J. Pharmacol.* **569**, 16–28.
- (9) Thati, B., Noble, A., Creaven, B. S., Walsh, M., Kavanagh, K., and Egan, D. A. (2007) An in vitro investigation of the induction of apoptosis and modulation of cell cycle events in human cancer cells by bisphenanthroline-coumarin-6,7-dioxacetatocopper(II) complex. *Chem.-Biol. Interact.* **168**, 143–158.
- (10) Hindo, S. S., Frezza, M., Tomco, D., Heeg, M. J., Hryhorczuk, L., McGarvey, B. R., Dou, Q. P., and Verani, C. N. (2009) Metals in anticancer therapy: copper(II) complexes as inhibitors of the 20S proteasome. *Eur. J. Med. Chem.* **44**, 4353–4361.
- (11) Bravo-Gomez, M. E., Garcia-Ramos, J. C., Gracia-Mora, I., and Ruiz-Azuara, L. (2009) Antiproliferative activity and QSAR study of copper(II) mixed chelate [Cu(N-N)(acetylacetonato)]NO₃ and [Cu(N-N)(glycinato)]NO₃ complexes, (Casiopinas). *J. Inorg. Biochem.* **103**, 299–309.
- (12) Mejia, C., and Ruiz-Azuara, L. (2008) Casiopinas IIgly and IIIia induce apoptosis in medulloblastoma cells. *Pathol. Oncol. Res.* **14**, 467–472.
- (13) De Vizcaya-Ruiz, A., Rivero-Muller, A., Ruiz-Ramirez, L., Kass, G. E., Kelland, L. R., Orr, R. M., and Dobrota, M. (2000) Induction of apoptosis by a novel copper-based anticancer compound, casiopina II, in L1210 murine leukaemia and CH1 human ovarian carcinoma cells. *Toxicol. in Vitro* **14**, 1–5.
- (14) Koiri, R. K., Trigun, S. K., Dubey, S. K., Singh, S., and Mishra, L. (2008) Metal Cu(II) and Zn(II) bipyridyls as inhibitors of lactate dehydrogenase. *BioMetals* **21**, 117–126.
- (15) Xie, H., Valera, V. A., Merino, M. J., Amato, A. M., Signoretti, S., Linehan, W. M., Sukhatme, V. P., and Seth, P. (2009) LDH-A inhibition, a therapeutic strategy for treatment of hereditary leiomyomatosis and renal cell cancer. *Mol. Cancer. Ther.* **8**, 626–635.
- (16) Singh, P., and Bhardwaj, A. (2008) Mechanism of action of key enzymes associated with cancer propagation and their inhibition by various chemotherapeutic agents. *Mini Rev. Med. Chem.* **8**, 388–398.
- (17) Kim, K. K., Lange, T. S., Singh, R. K., and Brard, L. (2010) Lipophilic aroylhydrazone chelator HNTMB and its multiple effects on ovarian cancer cells. *BMC Cancer* **10**, 72.
- (18) Lange, T. S., Zou, Y., Singh, R. K., Kim, K. K., Kristjansdottir, K., Sholler, G. L., and Brard, L. (2010) Chemotherapeutic effect of calceol derivative B3CD in a neuroblastoma xenograft model. *Chem. Biol. Drug Des.* **76**, 164–173.
- (19) Chagas, da, Silveira, V., Luz, J. S., Oliveira, C. C., Graziani, I., Ciriolo, M. R., and Maria da Costa Ferreira, A. (2008) Double-strand DNA cleavage induced by oxindole-Schiff base copper(II) complexes with potential antitumor activity. *J. Inorg. Biochem.* **102**, 1090–1103.
- (20) Ding, W.-Q., Yu, H.-J., and Lind, S. E. (2008) Zinc-binding compounds induce cancer cell death via distinct modes of action. *Cancer Lett.* **271**, 251–259.
- (21) Sigman, D. S., Bruce, T. W., Mazumder, A., and Sutton, C. L. (1993) Targeted chemical nucleases. *Acc. Chem. Res.* **26**, 98–104.
- (22) Sen, S., Mitra, S., Kundu, P., Saha, M. K., Krüger, C., and Bruckmann, J. (1997) Synthesis, characterization and structural studies of mono- and polynuclear complexes of zinc(II) with 1,10-phenanthroline, 2,2'-bipyridine and 4,4'-bipyridine. *Polyhedron* **16**, 2475–2481.
- (23) Dixit, N., Goto, K., Mishra, L., and Roesky, H. W. (2010) Supramolecular architectures constructed with the skeletons of zinc(II) 2,2'-bipyridine and barbiturate anion: Synthesis and characterization. *Polyhedron* **29**, 1299–1304.
- (24) Sorenson, C. M., and Eastman, A. (1988) Mechanism of cis-diamminedichloroplatinum(II)-induced cytotoxicity: Role of cell cycle arrest and DNA double strand breaks. *Cancer Res.* **48**, 4484–4488.
- (25) Bogdanović, et al., (2002) Growth effects of some platinum(II) complexes with sulfur-containing carrier ligands on MCF7 human breast cancer cell line upon simultaneous administration with taxol. *Metal Based Drugs* **9**, 33–43.
- (26) Tacka, K. A., Szalda, D., Souid, A.-K., Goodman, J., and Dabrowiak, J. C. (2004) Experimental and theoretical studies on the pharmacodynamics of cisplatin in Jurkat cells. *Chem. Res. Toxicol.* **17**, 1434–1444.
- (27) Arias, M. A., González-Alvarez, M., Fernández, M. J., Lorente, A., Alzuet, G., and Borrás, J. (2009) Synthesis and copper-mediated nuclease activity of a tetracationic tris(2, 2'-bipyridine) ligand. *J. Inorg. Biochem.* **103**, 1067–1073.

TX100126F