Ovarian Cancer Activity of Cyclic Amine and Thiaether Metal Complexes

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A thiaether metal complex 1-aza-4,7-dithiacyclononane-$RhCl_3$, **2**, and cyclic amine metal complexes tacn−$CuBr_2$, **3**, and Me_3tacn−$RuCl_3$, **4**, have been evaluated for anticancer activity against the ovarian cancer cell line NuTu-19 and for cell toxicity against the noncancerous ovarian tissue cell line OVepi. Specifically, metal complex **2** is active when compared to cisplatin at micromolar concentrations using the MTT and cell invasion assay. The in vitro results reported warrant further evaluation of metal complex **2** in living systems.

Introduction

Each year approximately 23 000 women are diagnosed with ovarian cancer, while only about half of this growing population can expect to survive the disease past 5 years.[1] At present, the standard treatment of ovarian cancer is cytoreductive surgery followed by intravenous chemotherapy involving platinum-based drugs. Though treatment of this disease is possible, the majority of the patients relapse leading to death. Therefore, successful treatment of ovarian cancer vitally depends on the discovery of more effective chemotherapeutic agents than the currently available platinum-based compounds.[2]

Research efforts focused on the use of nonplatinum-based anticancer agents have led to the synthesis of the metallocene dichlorides and the dirhodium carboxylates.[3,4] Each class of compound has shown anticancer activity, including titanocene, which proceeded into clinical trials. However, neither metallocene dichlorides nor dirhodium carboxylates are as active as cisplatin. This was evident when the efficacy of titanocene was found to be too low to pursue as a viable drug past phase II clinical trials.

Reported here is a thiaether metal complex and two cyclic amine metal complexes that are nonplatinum-based and possess anticancer activity. The lead complex, a Rh(III) thiaether complex, possesses very impressive anticancer activity when compared to that of cisplatin.

Results and Discussion

Various metal complexes of the organic ligands 1,4,7-triazacyclononane (tacn), *N*,*N*′,*N*″-trimethyl-1,4,7-triazacyclononane (Me_3tacn), and 1-aza-4,7-dithiacyclononane were synthesized and the activity against the ovarian cancer cell line NuTu-19 was explored. Also, control studies to determine the in vitro cell toxicity of these complexes against noncancerous ovarian cells were performed utilizing the normal ovarian tissue cell line OVepi. The OVepi cell line was isolated through a procedure developed by the authors. The ovaries of euthanized Fischer 344 rats were surgically removed, and the outer layer of epithelial cells were isolated by trypsinization of each individual ovary. Cell lines were then grown from the ovarian epithelial cells that were isolated. Only the $RhCl_3$ complex of 1-aza-4,7-dithiacyclononane **2** (Figure 1), the $CuBr_2$ complex of tacn **3** (Figure 2), and the $RuCl_3$ complex of Me_3tacn **4** (Figure 3) have shown sufficient in vitro activity at micromolar concentrations against ovarian cancer.

Impressively, complex **2** is very active when compared to cisplatin using the MTT assay. Metal complex **2** was synthesized, as shown in Scheme 1, by dissolving 1-aza-4,7-thiacyclononane **1** in ethanol and adding this solution into a stirring solution of rhodium trichloride in ethanol. The solution was refluxed for 2 h, yielding a brown solid that was filtered and then washed with ethanol and ether. X-ray quality crystals were grown from a concentrated sample of water. The crystal structure of **2** is shown in Figure 1. These types of compounds were chosen due to their cis-halide geometry, similar to that of cisplatin and its square planar geometry. Being square planar, cisplatin possesses a Cl−Pt−Cl angle of approximately 90°. Compound **2** has three different Cl−Rh−Cl angles ranging from 89.9° to 94.2°, making them very similar to the Cl−Pt−Cl angle in cisplatin.

The following graphs shown in Figures 4 and 5 are representative of the results obtained for the in vitro anticancer activity and cell toxicity studies of metal complexes **2**−**4**. Figure 4 represents the data obtained via the MTT assay for metal complexes **2**−**4** against the ovarian cancer cell line NuTu-19, and Figure 5 represents the data obtained for these complexes against the noncancerous ovarian cell line OVepi. Cells were plated at 5000, 10 000, and 20 000 cells per well in triplicate using 96-well plates and allowed to incubate overnight. Following this incubation period, metal complexes **2**−**4** were added to their designated wells and allowed to incubate with the cells overnight. An MTT stock solution was prepared by dissolving MTT in PBS. The solution was then added to each well and incubated for approximately 4 h. Viable cells in a colony will cleave MTT to give a blue crystalline precipitate of formazan. A sodium dodecyl sulfate solution was added to each well to dissolve any formazan crystals that had accumulated, and the cells were incubated overnight. Absorbance concentrations were then read on a μ-Quant Biotek Instruments microplate reader. Water was also used in this study as a control because the metal complexes were added to the wells as aqueous solutions at 2−3 μM.

The MTT results in Figure 4 show that metal complexes **2**−**4** possess anticancer activity, and the results in Figure 5 show that these metal complexes possess cell toxicity to the noncancerous cells similar to cisplatin. Although each metal complex

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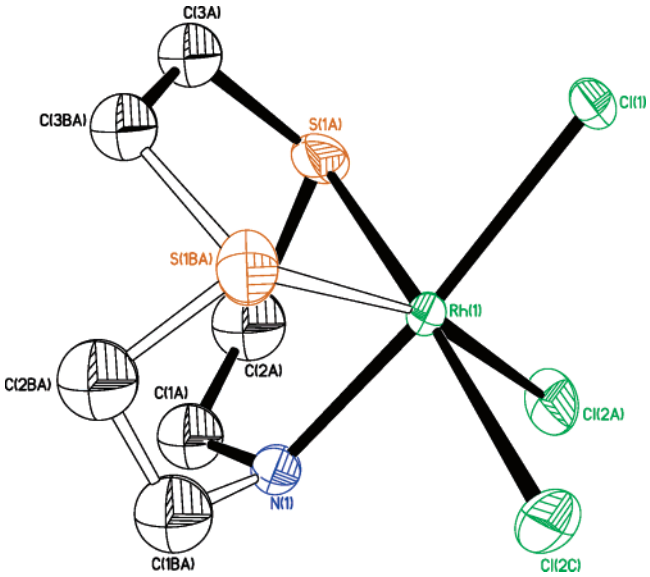


Figure 1. Thermal ellipsoid plot of **2** with thermal ellipsoids drawn at 50% probability. Hydrogen atoms have been omitted for clarity.

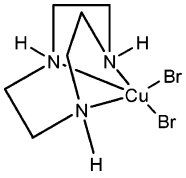


Figure 2. Metal complex tacn−$CuBr_2$ **3**.

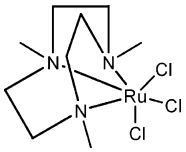
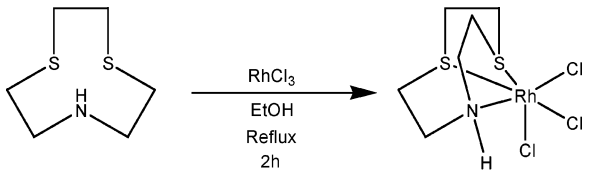


Figure 3. Metal complex Me_3tacn−$RuCl_3$ **4**.

Scheme 1. Synthesis of 1-Aza-4,7-dithiacyclononane−$RhCl_3$, **2**



1 **2**

exhibits anticancer activity, only **2** is as active as cisplatin, making it the most promising of the three after 24 h. However, after 3 days of testing, cisplatin becomes more effective.

The cell invasion assay[5] was also run against the NuTu-19 and OVepi cell lines to compare metal complex **2** and cisplatin by the procedure developed by Evans et al. The cell invasion assay utilizes the protein complex Matrigel which mimics the in vivo cellular basement membrane. Because it is necessary for cancer cells to degrade the basement membrane to invade and spread through the body, this assay explores how well compounds stop this degradation, keeping the tumor cells localized. Metal complex **2** and cisplatin were dissolved in the Matrigel at 10^{-4} and 10^{-6} M, 10^5 cells were plated in the top chamber of a two chamber cell invasion assay plate, and the cells were incubated for 48 h. Control assays were run with neither compound dissolved in the media and all assays were run in triplicate. After this incubation period, cell counts were conducted on the lower chamber by trypan blue staining to determine how many cells traversed through the Matrigel. The

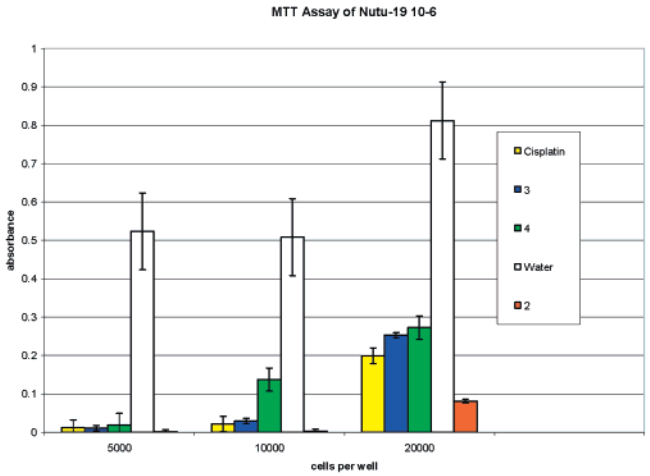


Figure 4. MTT assay results of metal complexes **2**−**4** vs the ovarian cancer cell line NuTu-19.

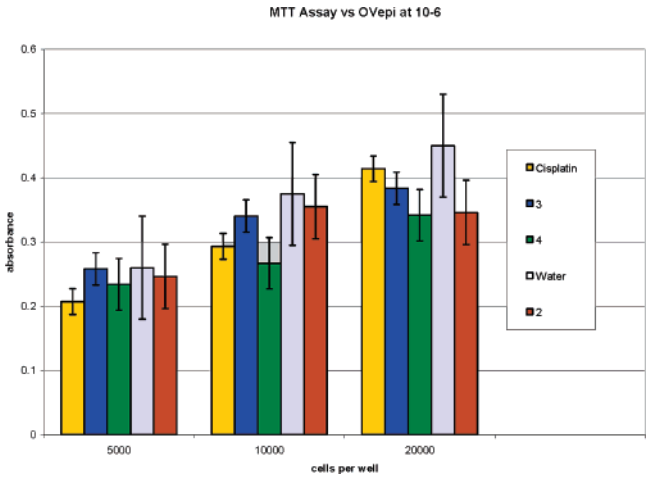


Figure 5. MTT assay results of metal complexes **2**−**4** vs the normal ovarian epithelial OVepi cell line.

lower the amount of cells that traverse through the Matrigel, the better the compound is at controlling the invasion of the cancer cells through the basement membrane mimic. The graphs in Figures 6 and 7 show the results obtained from these studies. These two graphs are presented as the amount of cells that traversed through the basement membrane with **2** and cisplatin dissolved in the Matrigel compared to the control assays where no compound was dissolved in the Matrigel. Compounds are considered effective agents when they show a large decrease in the number of cells traversing through the Matrigel when compared to the control for the cancerous NuTu-19 cells. While **2** and cisplatin both possess this characteristic, metal complex **2** appears to show a slight decrease in invasion when compared to cisplatin in the three trials that were run.

The results obtained in Figure 6 indicate that **2** is more efficient than cisplatin at decreasing the invasion of the cancerous cells through the basement membrane. Complex **2** allows approximately 25% invasion, while cisplatin allows about 35%.

The results obtained in Figure 7 indicate that **2** continues to allow the normal cell activity through the Matrigel better than cisplatin, especially at higher concentration.

Conclusion

In conclusion, we have shown that two cyclic amine metal complexes possess anticancer activity in vitro, an area of

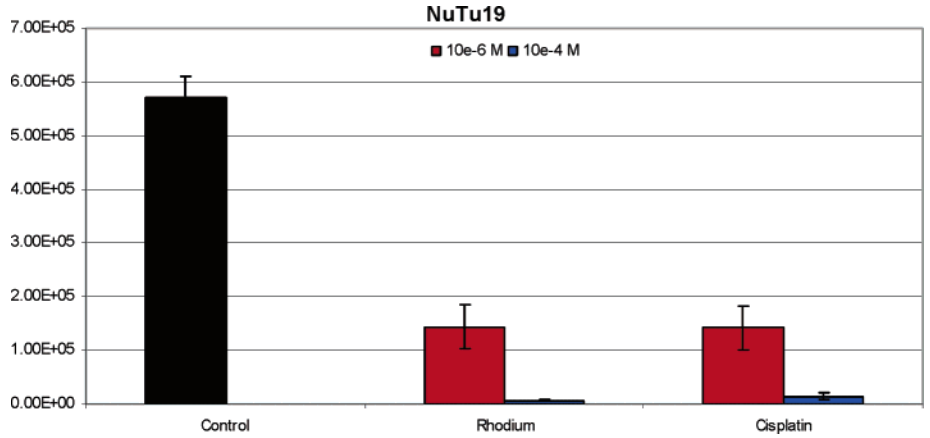


Figure 6. Cell invasion assay results of **2** (labeled rhodium) and cisplatin against NuTu-19.

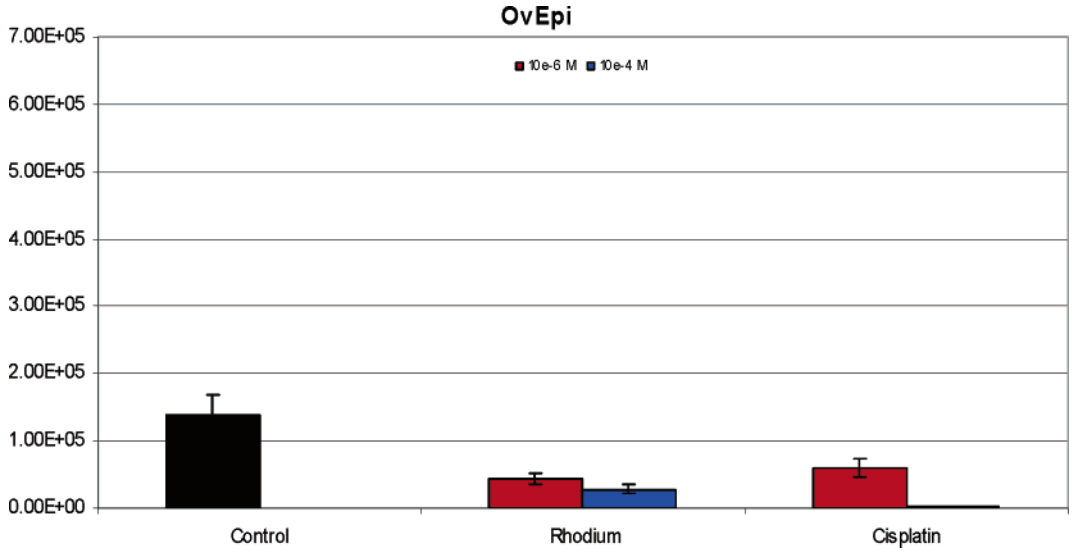


Figure 7. Cell invasion assay results of **2** (labeled rhodium) and cisplatin against OVepi.

research that has not previously been reported. We have also synthesized a Rh(III) metal complex, **2**, that possesses impressive anticancer activity, as well as cell invasion properties when compared to cisplatin at micromolar concentration. These findings have encouraged us to pursue more in-depth experiments involving the most promising of these metal complexes, **2**.

We are presently exploring the mechanism of action through DNA binding studies. Our goals are to understand if the mode of action is similar to that of cisplatin and, if so, which DNA bases are being complexed. Other in vitro studies, including the TUNEL assay and clonogenic assays, are also going to be conducted on these metal complexes. In vivo studies are also being explored to determine the toxicity and anticancer efficacy in living systems.

Experimental

General. Triazacyclononane (tacn), *N*,*N*′,*N*″-trimethyl-1,4,7-triazacyclononane (Me_3tacn), 1-aza-4,7-dithiacyclononane, and $RuCl_2(DMSO)_4$ were synthesized according to published procedures.[6−8] The metal complexes tacn−$CuBr_2$ and Me_3tacn−$RuCl_3$ were also synthesized by published procedures.[9,10] All other chemicals were used as received without further purification.

Elemental analysis was performed at the Microanalysis Laboratory at the University of Illinois. ^{1}H and ^{13}C NMR spectra were obtained on a Varian 300 MHz instrument.

Cell Lines. The ovarian cancer cell line NuTu-19[11] was cultured in media consisting of RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin−streptomycin−fungizone. The normal ovarian cell line OVepi was taken from the ovaries of euthanized Fischer 344 rats through a procedure similar to published procedures.[12] Ovaries were surgically removed post-death and placed in a cell culture dish containing trypsin. The ovarian epithelial cells were then collected via centrifugation. The cells were then added to cell culture in their respective media. Cells were allowed to grow to confluency, after which they were passed and frozen down to develop a backstock of cells. Ovepi cell culture media consisted of EMEM supplemented with insulin, collagen IV, 10% fetal bovine serum, and 1% penicillin−streptomycin−fungizone.

MTT Assay. The Vybrant MTT Cell Proliferation Assay Kit was purchased from Molecular Probes and protocol was followed. Cell concentrations were plated at 5000, 10 000, and 20 000 cells per well in triplicate in 96-well plates and allowed to incubate overnight. Metal complexes were then administered as aqueous solutions at 2−3 μM and again the cells were incubated overnight. Following incubation, a 12 mM MTT stock solution in phosphate-buffered saline was prepared, and 10 μL of the solution was added to each well. The cells were then incubated for 4 h, after which 100 μL of sodium dodecyl sulfate solution was added and incubated for 18 h. Absorbance of the formazan was recorded at 570 nm on a μ-Quant Biotek Instruments microplate reader.

Cell Invasion Assay. The cell invasion assay was run similarly to the studies conducted by Evans et al.[5] Metal complex **2** and cisplatin were dissolved in cell media at the desired concentration and then dissolved in the Matrigel. Cells were then plated at 10^5 cells in the top chamber of the two-chamber Matrigel system.

The cells were allowed to incubate for 48 h. After the 48 h incubation period, trypan blue stain cell counts were performed on the lower chamber to determine the number of cells that traversed through the Matrigel.

1-Aza-4,7-dithiacyclononane−$RhCl_3$ (2). In a 50 mL 3-necked round-bottom flask equipped with a stir bar, 1-aza-4,7-dithiacyclononane **1** (0.10 g, 0.61 mmol) dissolved in 1.07 mL of ethanol was added to a stirred solution of rhodium trichloride (0.128 g, 0.61 mmol) in 3.22 mL of ethanol. The solution was refluxed at 70 °C for 2 h, yielding a brown solid. After cooling to room temperature, it was filtered twice to ensure no solid was left in the filtrate and washed with first ethanol and then ether (0.20 g, 88%). ^{1}H NMR (400 MHz, D_2O): δ 3.83 (m), 3.54 (m), 3.31 (m), 3.10 (m), 2.99 (s). ^{13}C NMR (300 MHz, DMSO): δ 36.04, 36.56 (CH_2−S), 52.12 (CH_2−N). Anal. Calcd. for $C_6H_{13}NS_2RhCl_3$: C, 19.35; H, 3.52; N, 3.76; Rh, 27.63. Found: C, 18.97; H, 3.63; N, 3.54; Rh, 27.70. Mp 265 °C; EI-MS m/e = 373 (M^+).

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Supporting Information Available: Crystallographic data for compound **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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