Preparation, Characterization, & Cytotoxicity of Carboxylate-Stabilized Rhenium(I) Complexes

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Abstract

Rhenium complexes in the form of $Re(CO)_3$(bipyridine)X (where X =a pentylcarbonate group or a carboxylate group) have been found to be selectively cytotoxic, or cause cell death, for cancer cells compared with normal human cells. Research in our group has explored a variety of carboxylate groups with the intent of identifying key characteristics that lead to selective cytotoxicity towards cancer cells. To date, that research has found simple straight chain carboxylate groups impart the most selectivity and cytotoxicity to such complexes. The specific research completed in this project includes the preparation and characterization of several new carboxylate-stabilized complexes of the form, $Re(CO)_3$(bipyridine)X, along with a study of the effect of such complexes on the viability of cancer and normal human cells lines. The selective cytotoxicity of the rhenium complexes are examined based on the variations in the composition and structure of the carboxylate groups.
Two separate methods were used to prepare $Re(CO)_3$(bipyridine)X complexes. The rhenium carboxylate complexes were prepared by refluxing $Re_2(CO)_{10}$ and bipyridine (or 1,10-phenanthroline) in an ester containing the desired carboxylate group. Some of the complexes were synthesized by acidolysis reaction of $Re(CO)_3$(bipyridine)-(pentylcarbonate) with the appropriate carboxylic acid. Recrystallization experiments were carried out to purify some complexes. Nuclear magnetic resonance (NMR) spectroscopy and infrared (IR) spectroscopy were used to characterize the complexes. Combustion analysis for the percent carbon, hydrogen, and nitrogen in the new complexes determined the purity of the new rhenium complexes. Pure samples of new complexes are being tested for their effect on the viability of both cancer cell lines and normal cell lines.

Introduction

- Rhenium pentylcarbonate complexes have been shown to be cytotoxic [1].
- Rhenium tricarbonyl complexes have great potential for therapeutic usage to minimize the toxic side effects of platinum-based chemotherapy drugs [2].
- Rhenium tricarbonyl complexes can be prepared through a simplified procedure and the products are highly stable.
- Rhenium complexes are currently being investigated in preclinical studies for their ability to be utilized in clinical therapeutic applications as a transition metal anticancer agent [3].
- Our research group has observed the cytotoxicity of carboxylate-stabilized rhenium complexes prepared from the pentylcarbonate group.
- This research project investigates the preparation, characterization, and testing of complexes of the form $Re(CO)_3$(bipyridine)X (X = pentylcarbonate or carboxylate group).
- Cytotoxicity assays will determine the effect of carboxylate-stabilized rhenium (I) complexes on the viability of cancer and normal cell lines.

Table 1. Cytotoxicity results. IC_{50} values for bpy-stabilized complexes.

Carboxylate	IC50 (mM) HSC-2	IC50 (mM) HH1	IC50HF1 / IC50HSC-2
CHO2-	0.031	0.16	5.2
CH3CO2-	0.021	0.21	10
CHF2CO2-	0.20	0.48	2.4

Table 2. Combustion analysis of $Re(C_7H_5O_2)(CO)_3(C_{12}H_8N_2)$ and $Re(Cl_2CH_3CO_2)$-$(CO)_3(C_{10}H_8N_2)$.

$Re(CO)_3$o-phenbenzoate (C22H13N2O5Re)		
Analysis	Theoretical	Actual %
C	46.23	43.89
H	2.30	2.38
N	4.90	4.93

$Re(CO)_3$bpydichloroacetate (C15H9N2Cl2O5Re)		
Analysis	Theoretical	Actual %
C	32.50	32.24
H	1.64	1.59
N	5.05	4.94

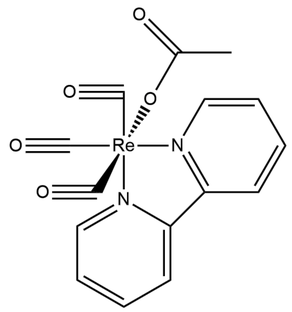

Figure 1. Chemical structure of $Re(CH_3CO_2)(CO)_3(C_{10}H_8N_2)$ (Re[acetate](CO)$_3$bpy).

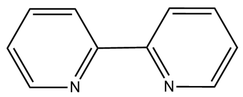

Figure 2. Chemical structure of 2,2'-bipyridine ligand (Bpy) ($C_{10}H_8N_2$).

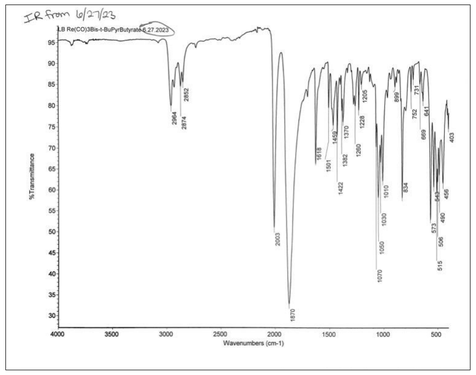

Figure 3. IR Spectrum of $Re(C_4H_7O_2)(CO)_3(C_9H_{13}N)_2$ (Re[butyrate](CO)$_3$(t-bu-pyr)$_2$).

Experimental

Acidolysis Reaction: $Re(O_2COC_5H_{11})(CO)_3(C_{10}H_8N_2)$
(Re(pentylcarbonate)(CO)$_3$bpy)

- Measured 50mg of Re(pentylcarbonate)$(CO)_3$bpy and added into a 50 mL Erlenmeyer flask containing a magnetic stir bar.
- Added 1 molar equivalent of formic acid = 3.4 µl of formic acid
- Added 5 mL of dichloromethane into flask. Stirred solution and observed color of solution.
- Micropipetted 5 µl (excess) of formic acid and added into stirring flask. Continued stirring the solution for 15 minutes.
- Added 10 mL of methyl *t*-butyl ether (MTBE) and 10 mL of pentane. Observed color of solution and presence of flocculent precipitate. Turned off stir plate after 15 minutes. Vacuum filtered the solid precipitate using a filter funnel and washed with MTBE. Weighed empty, labeled vial.
- Precipitate is dried and placed into the labeled vial for later use. Weighed the product and calculated percent yield.

Reflux Reaction: One-Pot Synthesis of $Re(CH_3CO_2)(CO)_3(C_{10}H_8N_2)$
(Re[acetate](CO)$_3$bpy)

- Measured 500 mg of $Re_2(CO)_{10}$. Measured 0.242 g of 2,2'-Bipyridine (Bpy). Measured 10 mL of phenyl acetate.
- Added all into a 50 mL one-necked round bottom flask containing magnetic stir bar. Utilized reflux setup. Greased stopcock. Connected flask to condenser and lowered flask into the heating mantle.
- Turned on stir plate. Ensured gas stopcock was disconnected from condenser to allow air to flow through condenser to flask.
- Turned on water for condenser and heating mantle. Heated solution for 24 hours.
- Reaction mixture cooled to room temp. Observed color of solution and presence of precipitate. Vacuum filtered the solid precipitate using a filter funnel and washed the flask and precipitate with pentane. Weighed empty, labeled vial.
- Precipitate is dried and placed into the labeled vial for later use. Weighed the product and calculated percent yield.

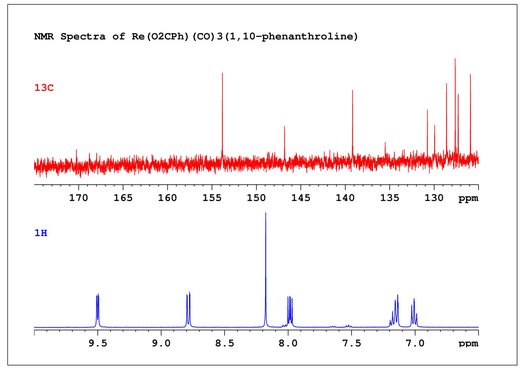

Figure 4. The Carbon (^{13}C) and Proton (^{1}H) NMR spectra for a sample of $Re(C_7H_5O_2)(CO)_3(C_{12}H_8N_2)$ {Re(benzoate)$(CO)_3$(o-phen)}.

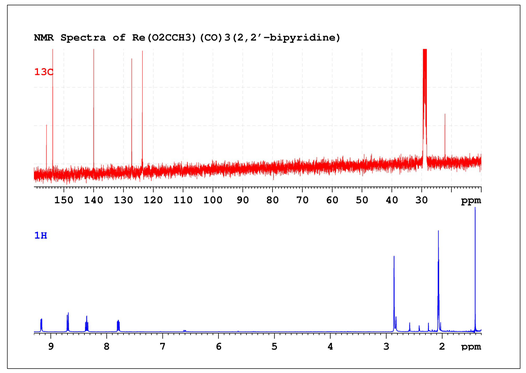

Figure 5. The Carbon (^{13}C) and Proton (^{1}H) NMR spectra for a sample of $Re(CH_3CO_2)(CO)_3(C_{10}H_8N_2)$ {Re(acetate)$(CO)_3$(bpy)}.

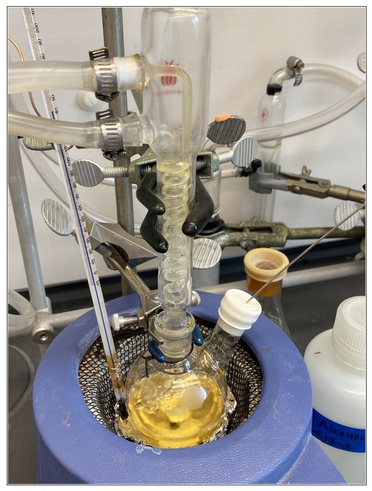

Figure 6. Reflux setup for the synthesis of a rhenium complex.

Results

- Rhenium complexes in the form of $Re(CO)_3$(bipyridine)X were prepared and characterized by IR and NMR spectroscopy.
- This lab found that the carboxylate-stabilized rhenium complexes with smaller carboxylate ligands are more selective and cytotoxic.
- Cytotoxicity assays completed on cancer cell line HSC-2 (human oral cancer) demonstrate that carboxylate-stabilized rhenium(I) complexes exhibit higher cytotoxic properties when the R group of the carboxylate ligand is both unbranched and small.
- The IC_{50} values increase as the length of the alkyl chain (R) increases (Table 1).
- The acetate ligand has the lowest IC_{50} value, which indicates that this ligand is the most cytotoxic complex.
- Reflux experiments that were performed to synthesize $Re(C_4H_7O_2)(CO)_3(C_{10}H_8N_2)$ (Re[butyrate](CO)$_3$bpy) produced an opaque, dark purple solution.

Conclusion

- The purple color of the reflux experiments of $Re(C_4H_7O_2)(CO)_3(C_{10}H_8N_2)$ indicates that the solution is not pure and contains a mixture of rhenium-containing complexes.
  - The purple color may indicate that the rhenium complex's bipyridine (bpy) ligand has adopted one extra electron, the thirteenth electron, that came from rhenium(0).
- When Re_2CO_{10} is refluxed with an ester that contains an aromatic ring, the aromatic ring is reduced, so that the reaction is successful and produces the product.
- The small carboxylate groups are cytotoxic because the carboxylate ligand is believed to readily leave the rhenium center and be replaced by water before entering the cell.
- Acetate becomes the counter ion to the rhenium complex cation because it is anionic and water is neutral.
- The rhenium complex is thought to migrate to the cell's mitochondria and cause the formation of reactive oxygen species (ROS) that attack the cancer cells which then undergo apoptosis.

References

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