SYNTHESIS, CHARACTERIZATION, AND ANTIMICROBIAL ACTIVITY OF ORGANO METALLIC RUTHENIUM(II) COMPLEXES

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Abstract
Six new organometallic ruthenium(II) complexes have been synthesized and characterized by elemental analyses and spectroscopic methods. They have been tested for their antimicrobial activity against various Gram(+) and Gram(-) bacteria. All the complexes are active against bacillus cereus and Salmonella typhimurium with complex [6], [(ηηηηη6-p-cymene)Ru(3-nb-TSC)Cl]PF6, being the most active. In general the complexes were no more active than the free ligands.

Keywords: ruthenium, thiosemicarbazone, diimine, organometallic, antimicrobial

Introduction

The key roles that transition metal ions play in biological systems and are well established. The discovery in the 1960’s of the anticancer properties of cis-diamminedichloroplatinum(II), cisplatin, revolutionized cancer chemotherapy (1). As a result, a large number of other metal coordination complexes have been screened for anticancer and antimicrobial properties. Of the other precious metals, much attention recently has been given to the chemistry of ruthenium (2-6). Ruthenium has unique properties which make it particularly useful in drug design. These properties include the rate of ligand exchange, the range of biologically accessible oxidation states and the ability of ruthenium to mimic iron in binding to certain biological molecules. Ruthenium complexes show antimicrobial activity as well (7-9). The activity of organic antimicrobials such as chloroquine (a drug used to treat malaria) has been enhanced by binding the organic molecule to a ruthenium center. The Ru(II)-chloroquine complex is 2-5 fold more effective than chloroquine alone.

Thiosemicarbazones (TSCs) (Figure 1) have received considerable attention because they present a wide range of bioactivities; antibacterial, antifungal, antitumoral, and antiviral (10). This is particularly applicable to heterocyclic and aromatic TSCs.

The biological properties of TSCs are often related to metal ion coordination. Although the free uncomplexed TSCs show interesting biological activity, in a number of cases the transition metal complexes of TSCs showed greater biological activity than the uncomplexed ligands (11, 12). This can be related to increased lipophilicity which controls entry into the cell. It has also been proposed that the mechanism of antibacterial activity involves electron transfer and/or oxidative stress (13). Other positive effects of metal coordination include potentially significant reduction of drug resistance (14) and side effects. It is conceivable that coordination to the metal serves to activate the biologically active TSC ligand.

In solution, organometallic compounds exhibit different ligand kinetics in comparison to coordination complexes. This is an important factor which could prove advantageous in the design of metal-based drugs. For instance, metallocenes of the type M(η5-C5H5)2X2 (M = Ti, V, Nb, and Hf) have shown moderate anticancer behavior (15). In this paper we report the synthesis of organometallic ruthenium complexes (ligands shown in Figure 2).

1. Part of this work was presented to the Arkansas Academy of Science.
and describe their characterization and antimicrobial activity.

**Experimental**

**Materials**

Analytical or reagent grade chemicals were used throughout. Hydrated RuCl$_3$ was purchased from Strem (Newburyport, MA) and used as received. All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO) or other commercial vendors and used as received. Microanalyses (C, H, N) were performed by Galbraith Laboratories (Knoxville, TN). $^1$H and $^{13}$C NMR spectra were recorded on a Varian Mercury spectrometer operating at 300 MHz in DMSO-$d_6$ or acetone-$d_6$. The chemical shifts were measured in ppm relative to TMS. IR spectra were recorded in KBr discs in the range 4000 – 450 cm$^{-1}$ on a Mattson Satellite FTIR spectrophotometer while the electronic spectra were recorded on an Agilent 8453 spectrophotometer in the range 190-1100 nm using quartz cuvettes. Melting points were determined in open capillaries and are uncorrected.

**Syntheses**

The precursor complex [(η$^6$-p-cymene)RuCl$_2$]$_2$ was prepared following a literature method (16). 1,10-phenanthroline-5,6-dione (phenedione) (17), dipyrido[3,2-α:2′,3′-c]quinoxaline (dpq), dipyrido[3,2-α:2′,3′-c](6,7,8,9-tetrahydro)phenazine (dpqC) (18), dipyrido[3,2-α:2′,3′-c]phenazine-11-carboxylic acid (dpq-CO$_2$H) (19) were also synthesized following literature protocols.

**Synthesis of [(η$^6$-p-cymene)Ru(dpqC)Cl]PF$_6$ [1]**

The dpqC ligand (220 mg, 0.77 mmol) was suspended in 50 mL of degassed MeOH. Under a flow of argon [(η$^6$-p-cymene)RuCl$_2$]$_2$ (230 mg, 0.376 mmol) was added to the yellow suspension resulting in an immediate color change to clear orange. The reaction mixture was heated at reflux for 30 min. The orange solution was filtered and solid NH$_4$PF$_6$ (270 mg, 1.66 mmol) was added to the filtrate. The solution volume was reduced to ~ 30 mL resulting in initiation of precipitation of an orange solid. The solution was placed in a freezer at -20 °C to complete the crystallization. The solution was filtered and the solid washed with 5-10 mL of ether. The bright yellow solid was dried in vacuo to give 0.40 g (80%).

**Synthesis of [(η$^6$-p-cymene)Ru(dpq)Cl]PF$_6$ [2]**

dpq (100 mg, 0.354 mmol) and NH$_4$PF$_6$ (128 mg, 0.785 mmol) was suspended in 30 mL of degassed MeOH. To the yellow suspension [(η$^6$-p-cymene)RuCl$_2$]$_2$ (99.8 mg, 0.163 mmol) was added under a flow of argon. The reaction mixture was heated at reflux for 40 min during which time a yellow-green solid precipitated. The reaction was allowed to cool to ambient temperature and then to -20 °C overnight. The yellow-brown solution was filtered and the solid washed with 50 mL of ether and dried in vacuo to give 0.21 g (91%) of the product.

**Synthesis of [(η$^6$-p-cymene)Ru(dppz-CO$_2$H)Cl]PF$_6$ [3]**

NH$_4$PF$_6$ (200 mg, 1.22 mmol) and [(η$^6$-p-cymene)RuCl$_2$]$_2$ (184 mg, 0.305 mmol) were dissolved in 30 mL of degassed MeOH. Under a flow of argon the dppz-CO$_2$H (200 mg, 0.609 mmol) was added in one portion. The reaction mixture was stirred at room temperature overnight during which time the orange solution changed to a yellow suspension. The mixture was filtered, the precipitate washed with 50 mL of ether and vacuum dried to give 0.37 g (84%) of the yellow product.

**Synthesis of [(η$^6$-p-cymene)Ru(dpq)Cl]PF$_6$ [4]**

NH$_4$PF$_6$ (526 mg, 3.22 mmol) and the dpq ligand (250 mg, 1.08 mmol) were suspended in 60 mL of degassed MeOH. Under a flow of argon [(η$^6$-p-cymene)RuCl$_2$]$_2$ (329 mg, 0.538 mmol) was added to produce a yellow-orange suspension. Under an inert atmosphere the reaction mixture was heated at reflux for 2 h. The suspension was cooled to ambient temperature and filtered to give a bright yellow solid. The solid was recrystallized from acetone/ether to yield 0.55 g of the product.
Synthesis of \([\eta^6-p\text{-cyocene}]\text{Ru(TSC)Cl}\text{PF}_6\) [5, 6]

The complexes with the thiosemicarbazone ligands were prepared as follows: \([\eta^6-p\text{-cyocene}]\text{RuCl}_2\text{2}(200 mg, 0.326 mmol) and the TSC (0.653 mmol) were dissolved in 25 mL of degassed MeOH and the solution stirred in an inert atmosphere overnight. To the red-orange solution was added NH_4PF_6 (213 mg, 1.31 mmol) and the solution stirred for a further 2 h. The solution was concentrated until precipitation began and then it was placed at -20 °C overnight. The solid that formed was filtered, washed with ether and recrystallized.

Antibacterial activity screens

The ligands, 3-nb-TSC and 3,4-dmb-TSC and the ruthenium complexes [1-6] were screened against standard bacterial strains Staphylococcus aureus, Salmonella typhimurium, Bacillus cereus, Proteus vulgaris and Pseudomonas aeruginosa (Carolina Biological). The antibacterial activity was studied by the disk diffusion method. Small, 7-mm diameter circles of filter paper (P5) were saturated with the test solutions (10^{-3} M in DMSO). The disks were placed on agar plates that were inoculated with the bacterial cultures and the plates incubated at 37 °C for 20 h. The starter cultures of the bacteria were grown overnight in nutrient broth. Chloramphenicol was used as a standard.

Results and Discussion

Syntheses

The starting ruthenium dimer, \([\eta^6-p\text{-cyocene}]\text{RuCl}_2\text{2}\) was made following the method of Bennett and Smith (16) by heating at reflux a methanolic solution of RuCl_3.xH_2O with α-phellandrene. From this precursor compound target complexes (using the ligands in Figure 2) were synthesized according to Scheme 1. Generally, the ruthenium dimer is reacted with two equivalents of the ligand in methanol at elevated temperatures for the diimine ligands and at room temperature for the TSCs. Ambient temperatures were used for the TSCs because a couple of reactions between the dimer and the TSCs at reflux temperatures led to the precipitation of a black shiny insoluble solid which we assume could be the metallic element. Under anaerobic conditions this would be due to the reduction of the Ru(II) center although we are
unaware of TSCs being able to reduce metal ions. Consider that the oxidation state of the metal center in half-sandwich organometallic complexes of the type $[(\eta^6-p$-cymene)Ru(Y)(Z)(X)]^+$ is stabilized by the arene which acts as a π-acid. With loss of the arene from the metal and having no other ligands in the system that can support the ruthenium in a low oxidation state, the metallic element may form.

The complexes were precipitated as the hexafluorophosphate salts by addition of NH$_4$PF$_6$. The complexes are insoluble in alcohols and water but are soluble in DMSO. They all gave satisfactory microanalyses given in Table 1. The analytical data are in good agreement with the proposed stoichiometry of the complexes.

**Vibrational spectra**

The infrared spectral data for the TSC ligands and their complexes are given in Table 2. Due to the presence of the –NH-C=S (thioamide) functional group, the TSC ligands can, in principle, exhibit thione ↔ thiol tautomerism (Figure 3).

![Figure 3. Thione (I) – thiol (II) tautomerism of TSCs](image)

The absence of the ν(S-H) band near 2570 cm$^{-1}$ in the IR spectra suggests that the thionic form of the ligand is present in the solid state (20). This is supported by the presence of a band at 3164 cm$^{-1}$ for 3-nb-TSC and 3181 cm$^{-1}$ for 3,4-dmb-TSC ascribable to the ν(N-H). The two bands in the region 3250-3450 cm$^{-1}$ are attributed to the stretching (symmetric and asymmetric) frequencies of the –NH$_2$ group. Both these groups are present in the metal complexes but are shifted somewhat. This implies that coordination to the metal occur through the azomethinic nitrogen and the thione sulfur. The shift of the ν(NH$_2$) bands is the expected consequence of the coordination of the sulfur from the S=C-NH$_2$. The ν(C=S) bands of the TSCs are sensitive to metal chelation providing evidence for metal coordination via the imine nitrogen (N$_1$). The band in 3-nb-TSC occurs at 1602 cm$^{-1}$ and shifts to 1623 cm$^{-1}$ in the complex. For 3,4-dmb-TSC there is a slight (5 cm$^{-1}$) shift on complex formation. The bands at ~850 cm$^{-1}$ which is attributable to the thioamide IV ν(C=S) band shift to lower energies by 10-18 cm$^{-1}$ in the complexes. The size of the shift is in agreement with coordination through the neutral ligand (21). The medium to weak vibration at ~1015 cm$^{-1}$ is assigned to ν(N$_1$-N$_2$) in agreement with other studies (22). This band is unaffected on complexation indicating that the N$_2$ atom does not participate directly in bonding to the metal.

**Electronic Spectra**

The electronic spectra of the ligands and their complexes were determined from 10$^{-5}$ M DMSO solutions in the region 190-820 nm. The data are shown in Table 3. The two TSCs showed a band below 220 nm which can probably be assigned to π-π* transitions; the bands between 330 and 400 nm may be assigned to n-π* transitions. The bands are bathochromically shifted by 20 nm and 15-30 nm respectively in the complexes. In particular the shift of the π-π* transition which is likely due to a weakening of the C=S bond is further indication of this moiety being linked to the metal center. In the TSC complexes a number of new bands are seen. These bands show up at 486 nm for [5] and 421 nm for the chloride analog of [6]. These bands are

<table>
<thead>
<tr>
<th>Complex</th>
<th>Empirical formula</th>
<th>Analyses, % found (calculated)</th>
<th>MP (°C)</th>
<th>Yield (%)</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C$<em>{28}$H$</em>{28}$N$_4$ClPF$_6$Ru</td>
<td>47.57(47.9) 4.07 (4.0) 8.00 (7.98)</td>
<td>266 dec.*</td>
<td>80</td>
<td>bright yellow</td>
</tr>
<tr>
<td>2</td>
<td>C$<em>{28}$H$</em>{24}$N$_4$ClPF$_6$Ru</td>
<td>46.79(48.17) 3.71(3.47) 7.85 (8.03)</td>
<td>180 dec.</td>
<td>91</td>
<td>yellow-green</td>
</tr>
<tr>
<td>3</td>
<td>C$<em>{28}$H$</em>{24}$N$_4$O$_2$ClPF$_6$Ru</td>
<td>45.92 (46.1) 3.68 (3.32) 7.46 (7.67)</td>
<td>201. dec.</td>
<td>84</td>
<td>yellow</td>
</tr>
<tr>
<td>4</td>
<td>C$<em>{24}$H$</em>{24}$N$_4$ClPF$_6$Ru</td>
<td>43.95 (44.49) 3.61 (3.42) 10.07 (8.65)</td>
<td>270 dec.</td>
<td>80</td>
<td>bright yellow</td>
</tr>
<tr>
<td>5</td>
<td>C$<em>{20}$H$</em>{27}$N$_3$ClO$_2$PF$_6$Ru</td>
<td>36.89 (36.67) 4.42 (4.15) 6.12 (6.42)</td>
<td>110</td>
<td>65</td>
<td>yellow</td>
</tr>
<tr>
<td>6</td>
<td>C$<em>{18}$H$</em>{22}$N$_4$O$_2$SClPF$_6$Ru</td>
<td>35.72 (33.78) 3.50 (3.47) 7.07 (8.75)</td>
<td>235 dec.</td>
<td>52</td>
<td>orange</td>
</tr>
</tbody>
</table>

* dec. = decomposed
moderately intense and are assigned as LMCT (S(σ,π → Ru(II)) transitions. The bands 630 nm and 637 nm could be MLCT or weak d-d transitions. With reference to previous work (24, 25), we assign the high energy bands (200-400 nm) for complexes [1 – 4] to intra-ligand π-π* transitions. Complexes [1] and [2] also show longer wavelength transitions (400-660 nm) that are then attributed to dπ-π* MLCT transitions that are common for Ru(II) polypyridyl complexes.

**Antimicrobial studies**

The *in vitro* antimicrobial properties of the metal complexes and the two TSCs were tested against Gram(+) and Gram(-) bacteria. The results are shown in Table 4. It was seen that all the tested compounds showed good cytotoxic activity against *Bacillus cereus* and *Salmonella typhimurium* (when compared to the chloramphenicol standard). With the exception of [6] which showed exceptional activity against *Staphylococcus aureus*, the other compounds displayed little activity against the other bacteria. Again with the exception of [6], the TSC ligands do not seem to be more or less active than their complexes.

**Table 2. Solution electronic spectral data (nm) for ruthenium arené complexes (10⁻⁵ M DMSO)**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Wavelength (molar absorptivity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-nb-TSC</td>
<td>208 (4.62) 318 (4.74) 398 (2.29)</td>
</tr>
<tr>
<td>3,4-dmb-TSC</td>
<td>333 (3.65) 345 (3.59)</td>
</tr>
<tr>
<td>Complexes</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.31 (4.40) 272 (4.48) 297 (sh) 657 (2.24)</td>
</tr>
<tr>
<td>2</td>
<td>230 (4.46) 282 (4.58) 388 (4.01) 487 (3.24) 6.57 (3.34)</td>
</tr>
<tr>
<td>3</td>
<td>232 (4.51) 293 (4.67) 376 (4.11) 397 (4.14)</td>
</tr>
<tr>
<td>4</td>
<td>232 (4.56) 267 (4.46) 355 (3.73)</td>
</tr>
<tr>
<td>5</td>
<td>232 (4.56) 348 (4.38) 486 (2.98) 657 (3.18)</td>
</tr>
<tr>
<td>6</td>
<td>232 (2.92) 342 (4.12) 630 (3.38)</td>
</tr>
</tbody>
</table>

*a 1 x 10⁻⁵ M in CH₃CN; b log ε; sh = shoulder*

**Table 3. Selected vibrational bands (cm⁻¹) of thiosemicarbazones and complexes [5] and [6]**

<table>
<thead>
<tr>
<th>Assignment</th>
<th>3-nb-TSC</th>
<th>3,4-dmb-TSC</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>υ (C=N)</td>
<td>1602</td>
<td>1618</td>
<td>1623</td>
<td>1623</td>
</tr>
<tr>
<td>υ (N-N)</td>
<td>1018</td>
<td>1018</td>
<td>1015 (w)</td>
<td>1015</td>
</tr>
<tr>
<td>υ (C=S)</td>
<td>1092</td>
<td>1097</td>
<td>1054</td>
<td>1091</td>
</tr>
<tr>
<td>υ (NH)</td>
<td>927</td>
<td>853</td>
<td>845</td>
<td>832</td>
</tr>
<tr>
<td>υ (NH₂)</td>
<td>3164</td>
<td>3181</td>
<td>3193</td>
<td>3230 (broad)</td>
</tr>
<tr>
<td></td>
<td>3433</td>
<td>3353</td>
<td>3396</td>
<td>3332</td>
</tr>
<tr>
<td></td>
<td>3281</td>
<td>3263</td>
<td></td>
<td></td>
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</tbody>
</table>

**Table 4. Antibacterial activity of the compounds – bacteriostatic diameter (mm)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bacterial strain</th>
<th>Gram (+)</th>
<th>Gram (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Bacillus cereus</em></td>
<td><em>Staphylococcus aureus</em></td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
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<td>&lt;1</td>
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<tr>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>&lt;1</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>4</td>
<td>&lt;1</td>
</tr>
<tr>
<td>3,4-dmb-TSC</td>
<td>&lt;3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3-nb-TSC</td>
<td>2</td>
<td>0</td>
<td>&lt;1</td>
</tr>
<tr>
<td>chloramphenicol</td>
<td>5</td>
<td>-</td>
<td>7</td>
</tr>
</tbody>
</table>
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References