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Organometallic Ruthenium Complexes of Novel Thiosemicarbazones

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Abstract.—We present the preliminary results of a study of two novel thiosemicarbazones (TSCs) and their ruthenium complexes. The TSCs were prepared by refluxing thiosemicarbazide with 9-anthraldehyde or benzanthrone in ethanol for 3 hours. The metal complex of each ligand (complex **I** = $[(\eta^6\text{-C}_6\text{H}_6)\text{Ru}(9\text{-ant-TSC})(\text{Cl})]\text{Cl}$ and complex **II** = $[(\eta^6\text{-C}_6\text{H}_6)\text{Ru}(\text{benz-TSC})(\text{Cl})]\text{Cl}$) was prepared by refluxing the appropriate TSC with $[(\eta^6\text{-C}_6\text{H}_6)\text{RuCl}_2]_2$. The compounds were characterized using infrared, ultraviolet-visible, and NMR spectroscopy. Two different methods, the disk diffusion test and luminometry, were used to test the compounds against a variety of different bacterial strains for antibacterial activity. The most optimistic results were obtained for the 9-ant-TSC ligand, especially in relation to activity against Gram (+) bacteria. The metal complexes showed no measurable activity and further biological testing of the metal complexes is currently being conducted.

Key words:— thiosemicarbazones (TSCs), ruthenium complexes, bacterial strains.

Introduction

In modern chemotherapy the aim is to use a chemical compound that kills the offending organism or cells while having minimal impact of other cells. While most of the current chemotherapeutic agents are organic compounds, the use of inorganic (defined as metal-based) compounds has been growing in importance over the last 30 years. Indeed one of the most important anticancer drugs is *cis*-diamminedichloroplatinum (II), cisplatin, which is especially useful for the treatment of solid malignancies. Cisplatin however exhibits serious renal toxicity and has a narrow-spectrum of activity (being applicable to only a few tumor types). This has led to a continuing effort to design transition metal-based drugs that improve on spectrum activity and are also less toxic when compared to cisplatin. In other biomedical spheres, metal-based compounds are also gaining prominence. For example, 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 (Dyson and Allardyce 2001).

Organometallic compounds exhibit different ligand kinetics in solution to coordination complexes, which could prove advantageous in the design of inorganic drugs. Metallocenes of the type $M(\eta^5\text{-C}_5\text{H}_5)_2\text{X}_2$ ($M = \text{Ti, V, Nb, and Hf}$) have shown moderate anticancer behavior (Clarke et al. 1999). Ru(II) arene complexes of the type $[(\eta^6\text{-arene})\text{Ru}(\text{XY})(\text{Z})]^+$ are cytotoxic to cancer cells including cisplatin-resistant cell lines (Morris et al. 2001, Aird et al. 2002).

Thiosemicarbazones (TSCs, Fig. 1) have received considerable attention because they present a wide range of bioactivities: antibacterial, antifungal, anti-neoplastic, and antiviral (Beraldo and Gambino 2004). They thus represent an important class of compounds that have aroused considerable interest in chemistry and pharmacology. The properties of TSCs are usually affected by metal coordination. Although the

free uncomplexed TSCs show interesting biological activity, in a number of cases the transition metal complexes showed greater biological activity (Pandeya and Dimmock 1993, Quiroga and Ranninger 2004). 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 (Kovacic et al. 1989). Other positive effects of metal coordination include potentially significant reduction of drug resistance and side effects (West et al 1991). It is conceivable that coordination to the metal serves to activate the biologically active TSC ligand. Also, the metal complex can exhibit different bioactivities than the free TSCs. By coupling the TSCs with the organometallic Ru(II) group, it may be possible to synthesize new complexes that have good biological activity due to the synergistic effectiveness.

In this paper we report the synthesis of organometallic ruthenium complexes with novel thiosemicarbazone ligands (Fig. 2) and describe their characterization and antimicrobial activity.

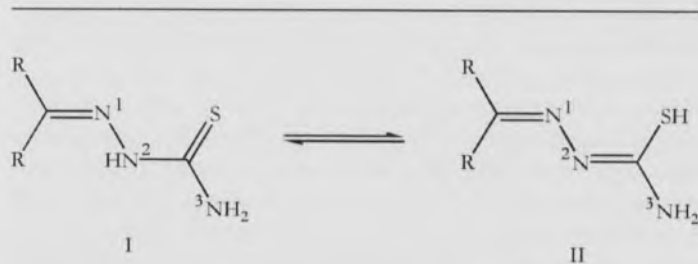


Fig. 1: Thiosemicarbazones showing thione (I) \rightleftharpoons thiol (II) tautomerism

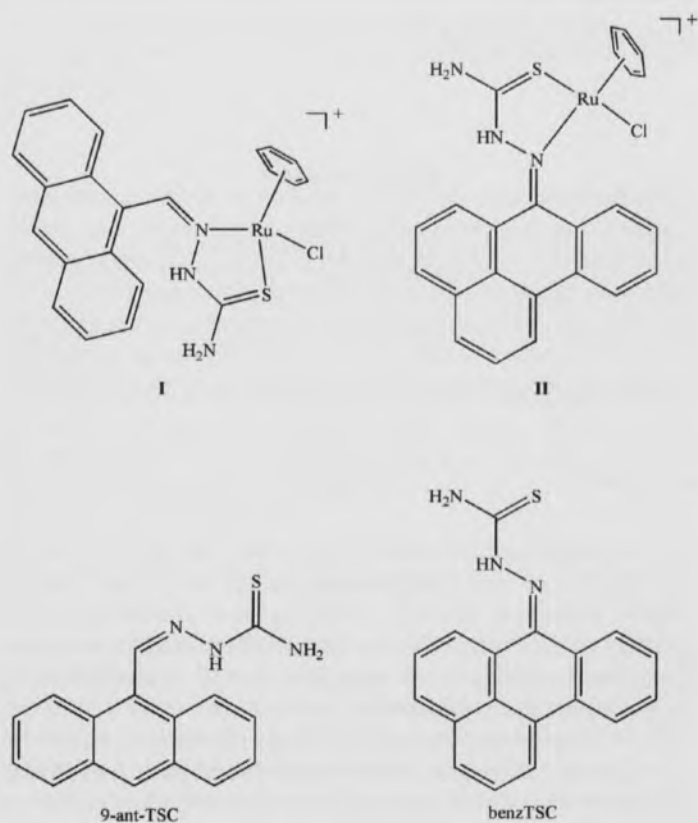


Fig. 2: Structures of the thiosemicarbazones and complexes

Materials and Methods

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. The luminometry studies were done using a BacTiter-Glo™ Microbial Cell Viability Assay (Promega, Madison, WI) on a Hidek Bioscan single-tube combination liquid scintillation counter and luminometer. Microanalyses (C, H, N) were performed by Desert Analytics (Tucson, AZ). ^1H and ^{13}C NMR spectra were recorded on a Bruker spectrometer operating at 300 MHz in chloroform- d_3 or dichloromethane- d_2 . 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, and 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. The precursor complex $[(\eta^6\text{-benzene})\text{RuCl}_2]_2$ was prepared following the method of Bennett and Smith (1974).

Preparation of TSC.—9-anthraldehyde (or benzanthrone)

was reacted with an equimolar amount of thiosemicarbazide in refluxing ethanol for 3 hours. The orange precipitate (yellow for benzanthrone) that formed was filtered, washed with copious amounts of ethanol, then ether, and dried at the vacuum pump. The yield was 82.7% for 9-ant-TSC and 43.4% for benz-TSC.

Preparation of complexes.—The complexes were prepared as follows: $[(\eta^6\text{-C}_6\text{H}_6)\text{RuCl}_2]_2$ (300 mg, 0.600 mmol) and the TSC (1.20 mmol) were dissolved in 40 mL of degassed toluene and the solution was stirred during reflux in an inert atmosphere for 4 hours. The brown precipitate (black for benzanthrone) was filtered, washed with copious amounts of pentane, and dried at the vacuum pump. The yield was 73.5% for **I** and 53.5% for **II**.

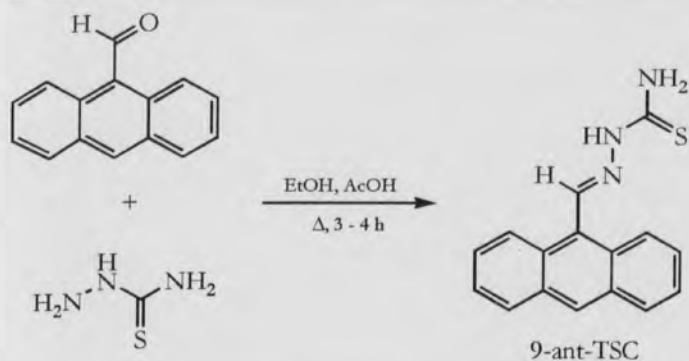
Antibacterial activity screens.—The ligands were screened against standard bacterial strains of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Enterococcus faecalis*, *Salmonella typhimurium*, and *Proteus vulgaris*. The antibacterial activity was studied by luminometry for the first 2 bacterial strains and by the disk diffusion method for the latter 4. For the luminescence assay, the bacteria were incubated in Mueller-Hinton broth at 37 °C for 20 hours. The cultures were diluted 1:100 in fresh M-H broth, and then 245 μl of the appropriate culture was added to 12 wells of a 24-well plate. Five microliters of the appropriate drug, 9-ant-TSC, benz-TSC, or chloramphenicol (the standard), all at 10^{-5} M were added to 4 wells of each culture, and the cultures were grown at 37 °C for an additional 5 hours. Luminescence was then measured as counts per second (CPS). For the disk diffusion test, small 7-mm-diameter circles of filter paper (P5) were saturated with 20 μl of 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 were incubated at 37 °C for 20 hours. Chloramphenicol was used as a standard. Following incubation, each plate was checked for zones of inhibition (measured in mm). Antibacterial activity screens for **I** and **II** using disk diffusion were also conducted, but the results were inconclusive.

Results and Discussion

Syntheses.—Figure 2 shows the ligands and complexes prepared in this study. The ligands were made by condensing the aldehyde (9-anthraldehyde) or ketone (benzanthrone) with thiosemicarbazide as shown in Scheme 1 for 9-ant-TSC. The starting ruthenium dimer $[(\eta^6\text{-benzene})\text{RuCl}_2]_2$ was made following the method of Bennett and Smith (1974) by heating at reflux a methanolic solution of $\text{RuCl}_3 \cdot x\text{H}_2\text{O}$ with 1,3- or 1,4-cyclohexadiene. From this precursor compound, target complexes (Fig. 2) were synthesized according to Scheme 2. Generally, the ruthenium dimer is reacted with two equivalents of the ligand in toluene at elevated temperatures. The complexes precipitated directly from the reaction solution on cooling to ambient temperature.

Melting Points.—Shown in Table 1 are the melting points for the TSCs and their complexes. The TSCs melted over a narrow

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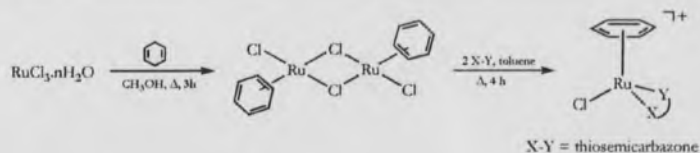


Scheme 1. Synthesis of the thiosemicarbazones

range (1.7-2.8°C), indicating relative purity of these compounds (as a melting range of about 2°C is generally considered normal for pure compounds). Neither complex showed any melting behavior below 350°C.

NMR Spectra.—The NMR data for the two ligands are consistent with the proposed structures. The ¹H NMR spectrum of 9-ant-TSC in CD₂Cl₂ shows two singlets at 9.00 ppm and 9.74 ppm that are assigned to the HC=N¹ and HN² protons respectively. The azomethinic (HN²) proton might be expected to show up at higher frequencies (11-14 ppm) but is shifted upfield in our spectrum. This is not unusual however as in a number of thiosemicarbazones the signal ascribed to this group show up in the range 8 – 11 ppm. The thione form of the ligand exists in solution as well as there is no peak at ~ 4.00 ppm in the ¹H NMR which corresponds to –SH. It has been reported that this resonance typically appears at ~ 4.0 ppm (Singh et al 2005). The aromatic resonances for both ligands appear at the expected positions (7.5 – 8.5 ppm). The ¹³C NMR spectra show a high frequency signal at 180.4 ppm for 9-ant-TSC and 184.1 ppm for benz-TSC. These are assigned to the –C=S moiety. The –C=N signal occurs at 141.4 ppm for 9-ant-TSC and 136.4 ppm for benz-TSC. In both compounds there is a cluster of peaks in the 120 – 130 ppm range, which is typical for aromatic compounds.

Electronic Spectra.—UV-Vis electronic spectra of both TSCs and complexes **I** and **II** were analyzed using 10⁻⁵ M DMSO solutions in the 190-900 nm region. Data from the analysis is presented in Table 1. The bands occurring at <300 nm can most likely be attributed to π-π* transitions, while the bands between 350-400 nm are most likely due to n-π* transitions of the thiosemicarbazone ligands. While the major absorbance wavelengths for the TSCs didn't change much (0-10 nm) on formation of their respective complexes, there were hypochromic changes at each peak (0.02-0.73). This clearly indicates electron density movement between the ligand and the metal. The π-π* transitions also show small changes in molar absorptivity (a general decrease of about 0.05 from the TSC to



Scheme 2. Synthesis of the metal complexes

the complex), which is likely a result of a weakening of the C=S bond. This indicates that the thione group is probably involved in metal coordination.

Vibrational Spectra.—The infrared spectral data for the ligands and complexes are shown in Table 2. Despite the fact that the HN=C=S group can undergo thione-thiol tautomerization (Fig. 1), the lack of a band around 2570 cm⁻¹ (characteristic of S-H bonds) indicates that the TSCs are in the thione form in the solid state. The presence of bands around 3150 cm⁻¹ indicates the presence of the –NH moiety, which further supports the thione coordination. These bands shift somewhat from the TSC to the related metal complex, possibly indicating involvement of the azomethinic nitrogen in complex formation. However, the N-N stretching (which occurs around 1015 cm⁻¹) does not vary much between TSC and the metal complex (2-7 cm⁻¹), which leads to the conclusion that the azomethinic nitrogen is not involved in formation of the complexes. Instead, the C=N band experiences a greater degree of shift between the TSC and metal complex (~14 cm⁻¹). Thus, it is concluded that the imine nitrogen is involved in metal coordination. The C=S bands for the TSCs is also shifted upon complex formation (from 827 to 809 cm⁻¹ for 9-ant-TSC and from 1279 to 1297 cm⁻¹ for benz-TSC), further indicating thione involvement in binding to the metal. The NH₂ bands show some amount of shift (as high as 24 cm⁻¹), but this occurred as a consequence of the coordination of the –S=C-NH₂ to the metal.

Antimicrobial Studies.—*In vitro* antibacterial properties of the TSCs are shown in Tables 3 and 4. Both TSCs showed little or no effect on Gram (-) bacteria, as determined from both the luminometry and disk-diffusion tests. In fact, no activity was seen for either TSC against the two Gram (-) bacteria in the disk-diffusion test, and both were about 4 times less effective against *E. coli* than against *S. aureus* in the luminometry assay. Benz-TSC also showed little effect against Gram (+) bacteria in the disk-diffusion test (0.5 mm), but had slightly higher effectiveness against *S. aureus* in comparison with 9-ant-TSC. Optimistic results for the 9-ant-TSC, especially in relation to Gram (+) bacteria, were obtained from the disk-diffusion test. Against *E. faecalis*, 9-ant-TSC was even more active than the chloramphenicol standard (bacteriostatic diameter of 10 mm, in comparison with 4 mm for the standard). Further studies are being done on this compound in an effort to establish minimum inhibitory concentration (MIC) values. Further biological

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Table 1. Solution Electronic Spectral Data (nm) for Selected Thiosemicarbazones and their Ruthenium Arene Complexes (10^{-5} M DMSO solutions)

Compounds	Wavelength (molar absorptivity)			
9-ant-TSC ^a (208.0 – 210.8°C) ^b	231 (4.53) ^c	264 (4.68)	373 (3.76)	392 (3.84)
I (>350°C)	231 (4.49)	263 (4.56)	375 (3.72)	393 (3.82)
benz-TSC (154.1 – 155.8°C)	231 (4.49)	266 (4.15)	276 (4.15)	393 (4.05)
II (>350°C)	231 (4.54)	262 (4.10)	398 (3.31)	405 (3.32)

^a = 1×10^{-5} M in DMSO; ^b = Melting Points; ^c = log (ϵ)Table 2. Selected Vibrational Bands (cm^{-1}) of Thiosemicarbazones and Complexes **I** and **II**

Assignment	9-ant-TSC	I	benz-TSC	II
ν (C=N)	1600	1614	1620	1622
ν (N-N)	1019	1017	1001	1008 (w)
ν (C=S)	1286	1285	1279	1297 (w)
	827	809 (w)	842	843
ν (NH)	3157	3146	3179	3168
ν (NH ₂)	3440	3417	3385	3386
	3263	3287	3264	3283

w = weak

Table 3. Antibacterial Activity of the Thiosemicarbazones and Complexes **I** and **II** (10^{-3} M) - Bacteriostatic Diameter (mm)

Compound	Bacterial strain			
	Gram (+)		Gram (-)	
	<i>Bacillus cereus</i>	<i>Enterococcus faecalis</i>	<i>Proteus vulgaris</i>	<i>Salmonella typhimurium</i>
9-ant-TSC	3	10	0	0
I	- ^a	-	-	-
benz-TSC	0.5	0.5	0	0
II	-	-	-	-
Chloramphenicol	9.5	4	4.5	8.5

^a = Results inconclusiveTable 4. Antibacterial Activity of the Thiosemicarbazones and Complexes **I** and **II** (10^{-3} M) - Luminescence (CPS)

Compound	Bacterial strain	
	Gram (+) <i>Staphylococcus aureus</i>	Gram (-) <i>Escherichia coli</i>
9-ant-TSC	1.30×10^7	5.72×10^7
I	- ^a	-
Benz-TSC	1.18×10^7	5.72×10^7
II	-	-
Chloramphenicol	3.22×10^6	3.39×10^6
Negative Control (Background)	1.13×10^5	1.13×10^5

^a = Results inconclusive

testing of the metal complexes is being conducted, but the preliminary results have been inconclusive.

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