

SYNTHESIS, CHARACTERIZATION AND CYTOTOXIC ANTIBACTERIAL ACTIVITY OF Ru(II) ETHOXY SALAL THIOSEMICARBAZONE COMPLEXES

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ABSTRACT

A set of complexes, [RuCl(CO)(B)(EPh₃)L] (where E=P/As; B=PPh₃/AsPh₃/py; L = ligand), of ruthenium have been synthesized by solution method. The structure of the ruthenium(II) complexes were determined by microanalytical techniques and spectral techniques (CHNS, Infrared, ultraviolet-visible and NMR spectroscopic techniques). FT-IR results reveal that thiosemicarbazone is coordinated to Ru(II) via ONS. In addition, the structure of the compounds, thiosemicarbazone ligand and complexes, was confirmed by Nuclear Magnetic Resonance Spectroscopy. Further, the synthesized ligand and its complexes were analyzed against human pathogens.

Keywords: Ethoxysalicylaldehyde, Thiosemicarbazide, Antibacterial activity, Ruthenium(II).

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INTRODUCTION

The synthesis of coordination complexes with mixed ligands have attracted widespread attention in the recent years due to their extensive biological properties i.e., antimalarial, antibacterial, anticancer, etc.¹ In the transition metals, noble group elements have shown significant contribution in the field of pharmacology. Among the noble group elements, ruthenium possesses variable oxidation states. Moreover, the ability to resemble the binding properties towards biological significance.² Schiff bases bearing N, O and S donor atoms are a kind of versatile ligands in coordination chemistry. Thiosemicarbazones are a class of ligands have emerged as relatively non-innocent ligands with varied biological properties.³ Thiosemicarbazone complexes derived from salicylaldehyde derivatives containing N₂O, N₂S, or ONS donor atoms have shown enormous impact in the pharmacology.⁴ In this present context, we have synthesized three ruthenium . These complexes were subjected to antibacterial activity.

EXPERIMENTAL

The solvents and chemicals were used are reagent grade and AR grade. The ethoxy derivative of salal thiosemicarbazone ligand was synthesized following a published procedure.⁵ The metal precursors, [RuHCl(CO)(py)(PPh₃)₂], [RuHCl(CO)AsPh₃)₃] and [RuHCl(CO)(PPh₃)₃], were synthesized by reported procedure.⁶⁻⁸ In a Vario EL III CHNS analyzer, microanalysis (elemental analysis) of the samples were performed. The Infrared spectrum and UV-Visible spectrum in DMSO solution of the samples were recorded in the range 4000-400 cm⁻¹ and spectrophotometer 2202 in the range 800-200 nm, respectively. In a Bruker AV III 500 MHz instrument, the ¹H NMR and ¹³C NMR spectra were recorded

using an internal reference (TMS). Similarly, ^{31}P NMR spectra were also recorded using orthophosphoric acid.

Synthesis of Ligand

The 1: the 1-mole ratio of 3-ethoxysalicylaldehyde in ethanol and thiosemicarbazide in methanol were added with continuous stirring to over a period of 1 h. The white colored precipitate was formed, filtered the white colored precipitate. Using TLC, the purity of the formed precipitate was checked.

Synthesis of Ligand : Ruthenium Complexes

The metal precursors (0.5mmol) in benzene was slowly added with stirring to a solution of ligand (0.5 mmol) in methanol and then refluxed for about 6 h. The resulted color solution was kept aside and formed precipitate is filtered off and subjected to purification by TLC.

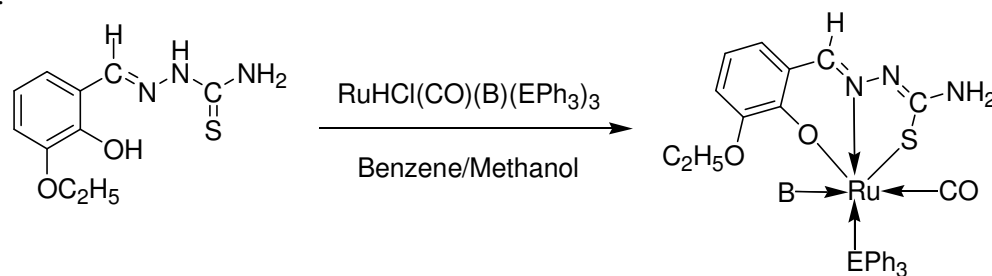
Antibacterial Activity

The standard agar diffusion method was used to evaluate the cytotoxic activity of the thiosemicarbazone ligand and the complexes against bacteria.²The data were collected as triplicate runs.

RESULTS AND DISCUSSION

Structure of the Complexes

Table-1 gives the results of analytical data and Table-2, 3 and 4 shows spectroscopic data (ligand & complexes) which reveals that a 1:1 metal-ligand stoichiometry was found. The synthesized complexes are stable in the environment. The synthesis and the proposed structure of the complexes are shown in Scheme-1.



E=P/As; B=PPh₃/AsPh₃/py

Scheme-1 : Synthesis of Ruthenium(II) Complexes

Table-1: Analytical Data

Ligand and Complexes	Color	M.Pt, °C	Elemental Analysis Calculated (Found) %			
			C	H	N	S
Ligand	White	260	50.19 (49.85)	5.48 (5.72)	17.56 (17.68)	13.40 (12.98)
[Ru(CO)(PPh ₃) ₂ L]	Brown	298	63.36 (63.38)	4.64 (5.03)	4.72 (5.11)	3.60 (3.65)
[Ru(CO)(AsPh ₃) ₂ L]	Red	290	57.67 (57.77)	4.22 (4.28)	4.29 (3.99)	3.28 (3.91)
[Ru(CO)(py)(PPh ₃)L]	Yellow	294	57.70 (57.81)	4.41 (4.41)	7.92 (8.02)	4.53 (4.76)

IR Spectra

IR spectroscopy is an important analytical technique in order to determine the coordination mode of the metal atom. The most important IR frequencies of the respective vibrations are tabulated in Table-2. The azomethine frequency of the complexes is observed at 1603-1611 cm⁻¹ which was shifted to a lower frequency than the free ligand at 1620 cm⁻¹. It confirms the coordination of the ligand to ruthenium *via* N.⁹ The $\nu(\text{C-S})$ vibrations assigned to 898 cm⁻¹ in the free ligand was shifted to lower vibrations at 765-783 cm⁻¹ ascribed to thiol nature of the ligand followed by coordination through the deprotonated sulfur.¹⁰ The band observed at 1213 cm⁻¹ is assigned to phenolic OH group. In complexes, it was disappeared and

a new band appeared at 1237-1249 cm^{-1} which reveals the other coordination mode through oxygen.¹¹ Further, in all the complexes studied the band at 1949-1963 cm^{-1} attributed to $\nu(\text{C}\equiv\text{O})$.¹² Overall, the free ligand is coordinated to ruthenium atom *via* ONS.

Table-2: FT-IR and Electronic Spectral Data

Ligand and Complexes	FT-IR, cm^{-1}					UV-vis, λ_{max} (nm)
	$\nu(\text{C}=\text{N})$	$\nu(\text{C}-\text{S})$	$\nu(\text{Ph}-\text{OH})$	$\nu(\text{Ph}-\text{CO})$	$\nu(\text{C}\equiv\text{O})$	
Ligand	1620	898	1213	-	-	301, 368
[Ru(CO)(PPh ₃) ₂ L]	1603	765	-	1246	1955	309, 369, 408
[Ru(CO)(AsPh ₃) ₂ L]	1611	783	-	1237	1949	308, 370, 410
[Ru(CO)(py)(PPh ₃)L]	1609	774	-	1249	1963	309, 371, 411

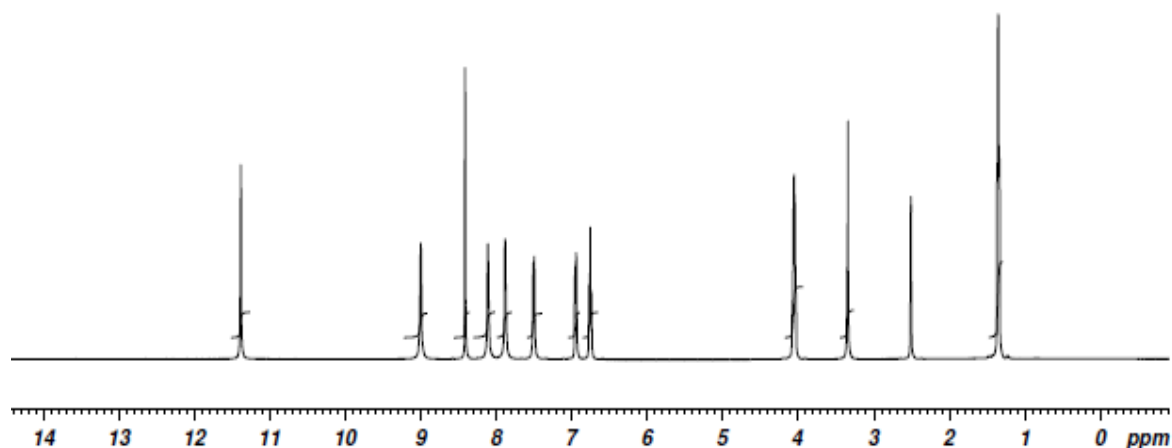
Electronic Spectra

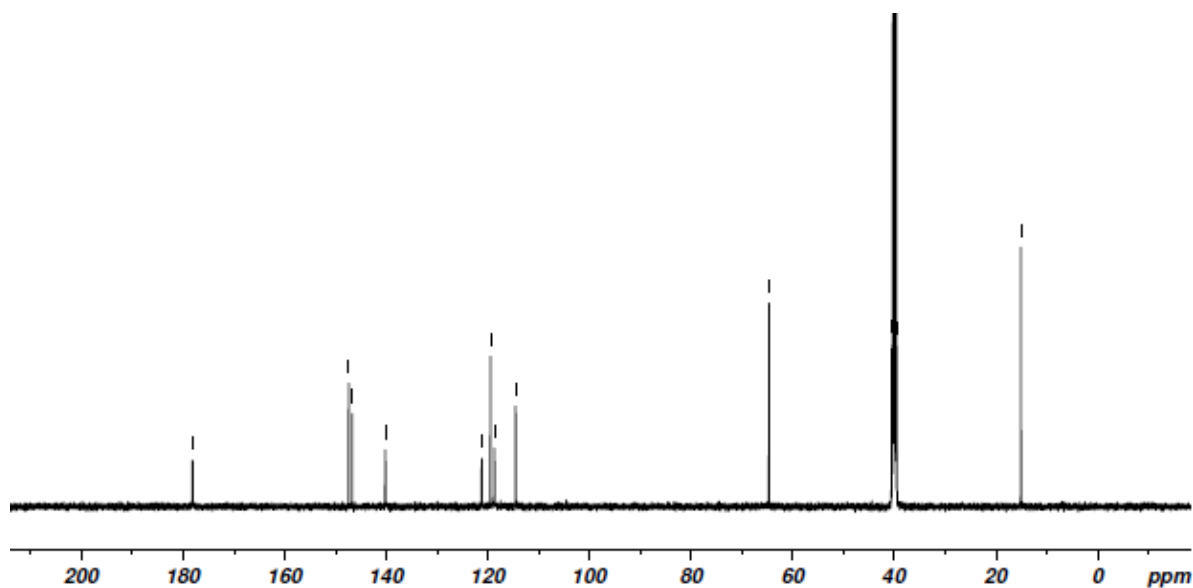
The UV-Visible spectral data were recorded in DMSO solvent and are tabulated in Table-2. The two bands of the ligand at 301 nm and 368 nm is assigned $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions. The thiosemicarbazone ligand bands were shifted to higher wavelength in complexes spectrum at 308-309 nm, 369-371 nm and a new band at 408-411 nm are assigned to charge transfer transitions. This confirms the coordination of thiosemicarbazone ligand to the ruthenium atom.¹³ The electronic spectral pattern of the synthesized complexes are similar to reported ruthenium(II) complexes.¹⁰

NMR Spectra

In order to confirm the binding mode of the thiosemicarbazone ligand to the ruthenium atom, the various NMR spectra were recorded in DMSO- d_6 . The chemical shift values of the compounds are listed in Table-3 and 4. The proton spectrum of the ligand shows a singlet at δ 9.0 due to phenolic OH proton. In complexes, it was disappeared which confirms the coordination through oxygen.¹⁴ The azomethine proton of the ligand was observed as a singlet at δ 11.3 in the free thiosemicarbazone ligand which was downfield in the spectra of the complexes at δ 11.5-11.8 ascribed the other coordination through azomethine N.¹⁵ The aromatic protons of the ligand and the complexes were appeared as multiplet in the range δ 6.6-8.6. The other methyl and methylene protons were showed at δ 1.3 and δ 3.3-3.5 respectively.

The aromatic carbon signals of the thiosemicarbazone ligand and its complexes showed in the region δ 110-139. The signal of the azomethine carbon in the ligand was detected at δ 147, downfield of this signal in the complexes at δ 141-145 supports the coordination through azomethine carbon.¹⁶ The signal at δ 178 corresponds to C=S of the free thiosemicarbazone ligand. It was disappeared in the complexes spectrum and new signal found at δ 169-172 confirms the coordination of sulphur.¹⁷ In all the complexes signal of the C \equiv O was observed at δ 192-196. The other carbon signals in the ligand and complexes due to methyl and methylene groups were displayed at δ 15-17, and δ 59-64, respectively.

Fig.1 ¹H NMR Spectrum of the Ligand

Fig.2 ^{13}C NMR Spectrum of the Ligand

The ^{31}P NMR spectrum of the complexes, $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)\text{L}]$ and $[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}]$, showed a signal at δ 35.2, and δ 36.3, respectively, due to the presence of PPh_3 (triphenylphosphine group). The NMR spectral analysis confirms the coordination of thiosemicarbazone ligand to the ruthenium atom.

Table-3: ^1H and ^{31}P NMR Data

Ligand and Complexes	^1H NMR data, δ					^{31}P NMR Data, δ
	Ph-OH	HC=N	Aromatic	CH_2	CH_3	PPh_3
Ligand	9.0	11.3	6.7-8.4	3.3	1.3	-
$[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}]$	-	11.5	6.6-8.4	3.4	1.3	36.3
$[\text{Ru}(\text{CO})(\text{AsPh}_3)_2\text{L}]$	-	11.8	6.8-8.6	3.4	1.3	-
$[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)\text{L}]$	-	11.6	6.6-8.5	3.5	1.3	35.2

Table-4: ^{13}C NMR data

Ligand and Complexes	Aromatic Carbon	C=N imine	C=S	C-S	C=O	CH_2	CH_3
Ligand	114-146	147	178	-	-	64	15
$[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}]$	110-135	143	-	171	194	60	16
$[\text{Ru}(\text{CO})(\text{AsPh}_3)_2\text{L}]$	120-139	145	-	169	192	62	15
$[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)\text{L}]$	119-137	141	-	172	196	59	17

Antibacterial Activity

To evaluate the cytotoxic activity, it is considered to study the antimicrobial activity. The results of the antibacterial activity are tabulated in Table-5.

Table-5: Antibacterial Activity

Ligand and Complexes	The diameter of Inhibition Zone (mm)	
	<i>S. aureus</i>	<i>E. coli</i>
Ligand	13	16
$[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}]$	19	22
$[\text{Ru}(\text{CO})(\text{AsPh}_3)_2\text{L}]$	18	19
$[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)\text{L}]$	21	24
Ciprofloxacin	24	27
DMSO	No activity	

The ligand showed significant zone inhibition data against Bacteria. The zone inhibition data of the complexes were higher than that of ligand. It confirms that the cytotoxicity can be increased by the coordination of the free thiosemicarbazone ligand to ruthenium atom.¹⁸ Though the complexes showed considerable antibacterial activity none of them could reach the potential of the standard drug.

CONCLUSION

In this present study, we have synthesized three ruthenium(II) complexes of 3-ethoxy salicylaldehyde thiosemicarbazone ligand. The characterization results of the complexes confirm the octahedral geometry of ruthenium atom with dibasic tridentate thiosemicarbazone ligands. All the complexes and ligand shows significant antibacterial activity. Comparatively, the ruthenium(II) complexes were shown higher activity. This clearly states that the coordination of thiosemicarbazone ligand to ruthenium atom modifies the biological properties of the ligand. All the complexes have higher growth inhibition activity against *E. coli* than the *S. aureus*.

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