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Synthesis, Characterisation and Antiamoebic Activity of New Thiophene-2-carboxaldehyde Thiosemicarbazone Derivatives and Their Cyclooctadiene Ru(II) Complexes

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Abstract—Reaction of new thiosemicarbazones (**1**–**4**) derived from thiophene-2-carboxaldehyde and cycloalkylaminothiocarbonylhydrazine with $[Ru(\eta^4\text{-}C_8H_{12})(CH_3CN)_2Cl_2]$ leads to form complexes (**1a**–**4a**) of the type $[Ru(\eta^4\text{-}C_8H_{12})(TSC)Cl_2]$ (where TSC = thiosemicarbazone). All the compounds have been characterised by elemental analysis, IR, ^1H NMR, electronic spectra and thermogravimetric analysis. It is concluded that the thionic sulphur and the azomethine nitrogen atom of the ligands are bonded to the metal ion. In vitro antiamoebic screening against (*HK-9*) strain of *Entamoeba histolytica* indicated that the Ru(II) complexes of thiophene-2-carboxaldehyde thiosemicarbazones were found more active than the thiosemicarbazones.

Entamoeba histolytica is estimated to infect more than 10% of the world's population. Approximately 50–100 million people develop invasive colitis and liver abscess,[1] which leads to 100,000 deaths per annum.[2] Metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole] is considered to be the drug of choice, which has common side effects.[3] It is mutagenic in bacteria[4] and potentially carcinogenic but has never been conclusively linked to the development of human malignancy.[5] Metronidazole affects electron transport and the chemically reactive, reduced form of metronidazole is cytotoxic to parasites.[6] There have been few reports of resistance of *E. histolytica* to metronidazole.[7] It is desirable, therefore, to find new amoebicides for a greater margin of safety and have amoebicidal activity with less toxicity for the host. Thiosemicarbazone derivatives have a great importance in chemistry and biology due to their antiprotozoal,[8] antibacterial,[9] antiviral,[10] and antineoplastic[11] activities. The metal thiosemicarbazone complexes exhibit a number of relevant biological properties.[12] The ligands coordinate to metal ions inside the cell, forming complexes which supposedly act as the true active species. In this sense, the behaviour of the ligands can be modified by the linkage to metal ion enhancing their biological activity.[13] The Ru complexes have been shown to have a greater sensitising efficiency, lower toxicity, and a lower reaction rate with non-protein sulphydryls than the corresponding free ligands. $[RuCl_2(DMSO)_2(4\text{-nitroimidazole})_2]$ has been used successfully as radiosensitiser. Earlier studies on Ru complexes such as *cis*-$RuCl_2(DMSO)_4$, as antineoplastic agents, have suggested a DNA binding mechanism.[14] The more electron affinic Ru complexes are always less toxic then their corresponding ligands. This phenomenon has been observed in another class of nitroimidazoles,[15] but can probably be attributed to the redox potential of the Ru metal. Additional advantages of Ru are the availability of both the Ru(II) and Ru(III) oxidation states under physiological conditions and the general substitution inertness of their ions when coordinated to nitrogen ligands.[16]

Approaches using ruthenium as antiparasitic agents, the complex $RuCl_2(CTZ)_2$ (CTZ = clotrimazole) displays good activity against *Trypanosoma cruzi*,[17] while $RuCl_2(CQ)_2$ (CQ = chloroquine) is efficient against *Plasmodium falciparum*.[18] However, the potential of ruthenium complexes as antiamoebic agents has so far

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been explored very little.[19] The significant leishmanicidal activity[20] of hydrazones of thiophene-2-carboxaldehyde led us to study the screening of thiosemicarbazones and their ruthenium complexes as antiamoebic agents. We now report the preparation of new cyclic thiosemicarbazones of thiophene-2-carboxaldehyde (Fig. 1) and their 1,5-cyclooctadiene Ru(II) complexes. These compounds were tested in vitro for their ability to inhibit the growth of *E. histolytica*; it was found that the chelation induced significant changes in the antiamoebic activity.

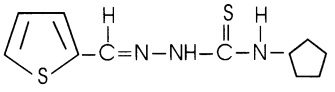
Chemistry

All the cycloalkylaminothiocarbonylhydrazines were prepared using the method reported by O'Sullivan[21] and their thiosemicarbazones were synthesised by refluxing the solution of cycloalkylaminothiocarbonylhydrazines (0.003 mol) in water (10 mL) and the solution of thiophene-2-carboxaldehyde (0.003 mol) in ethanol (10 mL) at 25 °C for 3 h with continuous stirring. After cooling, the crystals were filtered and recrystallised. The precursor used for the synthesis of Ru(II) complexes $[Ru(\eta^4\text{-}C_8H_{12})(CH_3CN)_2Cl_2]$ was synthesised by the literature procedure.[22] Melting point determination was carried out to check the purity of the compounds. Structures were confirmed by IR, ^{1}H NMR and electronic spectral studies. All 1,5-cyclooctadiene Ru(II) complexes were prepared by mixing an equimolar ratio of ligand and $[Ru(\eta^4\text{-}C_8H_{12})(CH_3CN)_2Cl_2]$ in refluxing methanol. The solution was kept at 0 °C overnight, the product was separated by filtration and finally washed with methanol. Recrystallisation was effected in methanol/DMF (8:2).

$$[Ru(\eta^4\text{-}C_8H_{12})(CH_3CN)_2C1_2] + TSC \xrightarrow[5\ h,\ reflux]{CH_3OH} [Ru(\eta^4\text{-}C_8H_{12})(TSC)Cl_2] + 2CH_3CN$$

where TSC = thiosemicarbazones **1**, **2**, **3** and **4**.

All Ru(II) complexes are soluble in DMF and DMSO, sparingly soluble in methanol, ethanol and insoluble in water. Analytical and spectral data (IR, electronic and ^{1}H NMR spectra)[23] are in good agreement with the composition of thiosemicarbazones **1**, **2**, **3** and **4** and their 1,5-cyclooctadiene Ru(II) complexes **1a**, **2a**, **3a** and



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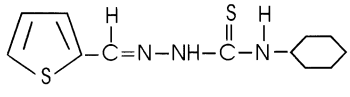

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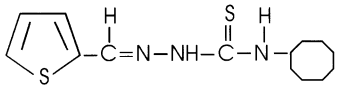

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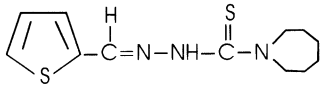

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Figure 1. 1. 2-TCA-CPTSC, thiophene-2-carboxaldehyde-cyclopentyl thiosemicarbazone; 2. 2-TCA-CHTSC, thiophene-2-carboxaldehyde-cyclohexyl thiosemicarbazone; 3. 2-TCA-COTSC, thiophene-2-carboxaldehyde-cyclooctyl thiosemicarbazone; 4. 2-TCA-HMTSC, thiophene-2-carboxaldehyde-hexamethylene thiosemicarbazone.

Table 1. Analytical and physicochemical data of new thiosemicarbazones and their Ru(II) complexes

S. no.	Compound/stoichiometry[a]	Colour	Yield (%)	Mp/dec. temp (°C)	Found (calcd)			
					C	H	N	Cl
1	2-TCA-CPTSC $C_{11}H_{15}N_3S_2$	Light yellow	54	140	52.71 (52.70)	6.14 (5.93)	16.81 (16.90)	
1a	$[Ru(\eta^4\text{-}C_8H_{12})$(2-TCA-CPTSC)$Cl_2]$ $C_{19}H_{27}N_3S_2Cl_2Ru$	Brown	61	250	42.31 (42.77)	5.25 (5.06)	7.98 (7.88)	13.10 (13.32)
2	2-TCA-CHTSC $C_{12}H_{17}N_3S_2$	Yellow	49	200	54.30 (53.93)	6.66 (6.36)	15.81 (15.73)	
2a	$[Ru(\eta^4\text{-}C_8H_{12})$(2-TCA-CHTSC)$Cl_2]$ $C_{20}H_{29}N_3S_2Cl_2Ru$	Brown	65	265	43.58 (43.87)	5.50 (5.30)	7.95 (7.67)	12.59 (12.97)
3	2-TCA-COTSC $C_{14}H_{21}N_3S_2$	Light yellow	51	150	57.15 (56.95)	7.36 (7.12)	14.45 (14.23)	
3a	$[Ru(\eta^4\text{-}C_8H_{12})$(2-TCA-COTSC)$Cl_2]$ $C_{22}H_{33}N_3S_2Cl_2Ru$	Brown	54	300	45.94 (45.91)	5.80 (5.74)	7.33 (7.30)	12.05 (12.35)
4	2-TCA-HMTSC $C_{12}H_{17}N_3S_2$	Yellow	63	115	54.01 (53.93)	6.50 (6.36)	15.39 (15.73)	
4a	$[Ru(\eta^4\text{-}C_8H_{12})$(2-TCA-HMTSC)$Cl_2]$ $C_{20}H_{29}N_3S_2Cl_2Ru$	Brown	67	300	43.33 (43.87)	5.58 (5.30)	7.88 (7.67)	13.05 (12.97)

[a]For abbreviations, see Figure 1.

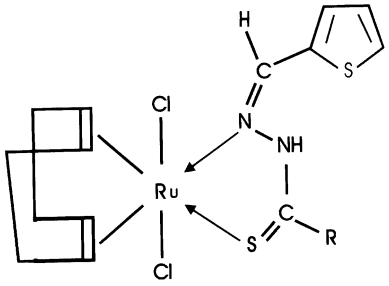

Figure 2. Structure of ruthenium(II) complexes (**1a**. R = –NHC_5H_9, **2a**. R = –NHC_6H_{11}, **3a**. R = –NHC_8H_{15}, **4a**. R = –NC_6H_{12}).

4a. Analytical and other physicochemical data of the compounds are presented in Table 1. In the IR spectra, the band due to ν(C–S–C) (ring) of thiophene moiety remains unaltered in **1a**, **2a**, **3a** and **4a** (Fig. 2), whereas the disappearance of the band due to NH–C=S upon complexation, and the appearance of a new band of azine nitrogen and the thiosulphur of the ligand along with the negative shift of 15–45 cm^{-1} of C=N band is observed in the complexes. It indicates the involvement of azomethine nitrogen in complexation.[24] This was supported by the shift of the N–N band of ligand on coordination. The broad band observed in region 3200 cm^{-1} may be due to ν(N–H) stretch being slightly shifted in complex. A strong band at 1057–1090 cm^{-1} ascribed to ν(C=S) of ligands is shifted to lower frequency (16–35 cm^{-1}), indicating the bonding of metal through thionic sulphur. The preferential coordination of thionic sulphur over sulphur of thiophene is due to the more nucleophilic character of the former.

In Vitro Antiamoebic Activity

The new thiosemicarbazones (**1**–**4**) and their complexes (**1a**–**4a**) were tested for amoebiasis in vitro against (*HK-9*) strain of *E. histolytica* by microdilution method.[25] Metronidazole was used as a reference amoebicidal drug. The biological test was carried out using DMSO as the solvent in which the compounds are stable. The in vitro antiamoebic activities of new thiosemicarbazones and their Ru(II) complexes are listed in Table 2. Metronidazole had a 50% inhibitory concentration (IC_{50}) against (*HK-9*) strain of *E. histolytica* ranging from 0.17 to 0.37 μg/mL.[26] It has been observed that the presence of certain bulky groups at position N^4 of the thiosemicarbazone moeity greatly enhances the activity.[27] In our experiment, compounds **1**, **2** and **3** showed activity by increasing the size of the cyclic ring, but compound **4** showed less activity than others. Most of the metal complexes that were investigated showed significant antiamoebic activity. The highest level of activity was exhibited by **2a**. The incorporation of metal ion enhanced the activity of the basic molecule. Detailed in vivo studies of these compounds are in progress.

Table 2. Antiamoebic activity of new thiosemicarbazones and their 1,5-cyclooctadiene ruthenium(II) complexes against (*HK-9*) strain of *E. histolytica*

S. No.	Compound	IC_{50} (μg/mL)	SD[a]
1	2-TCA-CPTSC	0.64	0.032
1a	[Ru(η^4-C_8H_{12})(2-TCA-CPTSC)Cl_2]	0.39	0.024
2	2-TCA-CHTSC	0.56	0.041
2a	[Ru(η^4-C_8H_{12})(2-TCA-CHTSC)Cl_2]	0.33	0.018
3	2-TCA-COTSC	0.50	0.027
3a	[Ru(η^4-C_8H_{12})(2-TCA-COTSC)Cl_2]	0.45	0.038
4	2-TCA-HMTSC	0.70	0.055
4a	[Ru(η^4-C_8H_{12})(2-TCA-HMTSC)Cl_2]	0.56	0.045
5	Metronidazole	0.22	0.050

[a]SD, Standard deviation.

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23. **1** ^{1}H NMR ($CDCl_3$) δ 10.39 (NH), 7.04–8.15 (m, 3H, aryl), 4.68 (m, 8H, CH_2); IR ν (cm^{-1}) 3170 (NH), 1598 (C=N), 1088 (C=S); λ_{max} (cm^{-1}) 30120, 38170, 48780. **2** ^{1}H NMR ($CDCl_3$) δ 10.06 (NH), 7.04–7.38 (m, 3H, aryl), 4.27 (m, 10H, CH_2); IR ν (cm^{-1}) 3165 (NH), 1600 (C=N), 1076 (C=S); λ_{max} (cm^{-1}) 30120, 38160, 48880. **3** ^{1}H NMR ($CDCl_3$) 9.87 (NH), 7.04–7.37 (m, 3H, aryl), 4.48 (m, 14H, CH_2); IR ν (cm^{-1}) 3154 (NH), 1560 (C=N), 1092 (C=S); λ_{max} (cm^{-1}) 30110, 38170, 48790. **4** ^{1}H NMR ($CDCl_3$) δ 8.96 (NH), 7.02–7.87 (m, 3H, aryl), 4.11 (m, 12H, CH_2); IR ν (cm^{-1}) 3110 (NH), 1598 (C=N), 1090 (C=S); λ_{max} (cm^{-1}) 25000, 30303, 37175, 43668. **1a** ^{1}H NMR ($CDCl_3$) δ 3.54 (m, 2H, CH), 2.61 (m, 4H, *exo* CH_2), 2.01 (m, 4H, *endo* CH_2), 7.01–7.99 (m, 3H, aryl); IR ν (cm^{-1}) 3370 (NH), 1600 (C=N), 1551 (C=C), 1057 (C=S), 472, 438 (Ru–N, Ru–S); λ_{max} (cm^{-1}) 41517, 30234, 25915. **2a** ^{1}H NMR ($CDCl_3$) δ 3.57 (m, 2H, CH), 2.62 (m, 4H, *exo* CH_2), 1.91 (m, 4H, *endo* CH_2), 6.98–7.61 (m, 3H, aryl); IR ν (cm^{-1}) 3200 (NH), 1624 (C=N), 1588 (C=C), 1070 (C=S), 500, 468, 442 (Ru–N, Ru–S); λ_{max} (cm^{-1}) 40816, 31645, 25000. **3a** ^{1}H NMR ($CDCl_3$) δ 3.25 (m, 2H, CH), 2.53 (m, 4H, *exo* CH_2) 1.87 (m, 4H, *endo* CH_2), 7.14–7.63 (m, 3H, aryl); IR ν (cm^{-1}) 3370 (NH), 1589 (C=N), 1554 (C=C), 1057 (C=S), 517, 478, 451 (Ru–N, Ru–S); λ_{max} (cm^{-1}) 40816, 33333, 29857, 25707. **4a** ^{1}H NMR ($CDCI_3$) δ 3.40 (m, 1H, CH), 2.6 (m, 4H, *exo* CH_2), 1.96 (m, 4H, *endo* CH_2), 7.16–7.60 (m, 3H, aryl); IR ν (cm^{-1}) 3300 (NH), 1622 (C=N), 1538 (C=C), 1058 (C=S), 480, 450 (Ru–N, Ru–S); λ_{max} (cm^{-1}) 26525, 41067.

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