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Synthesis and Antiamoebic Activity of New Cyclooctadiene Ruthenium(II) Complexes with 2-Acetylpyridine and Benzimidazole Derivatives

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Abstract—Reaction of [Ru(η^4-C_8H_{12}) $(CH_3CN)_2$ Cl_2] with 2-(2-pyridyl) benzimidazole or Schiff bases derived from 2-acetylpyridine and *S*-methyldithiocarbazate, *S*-benzyldithiocarbazate and thiosemicarbazide leads to form new complexes of the type [Ru(η^4-C_8H_{12})(L)Cl_2] (where L = ligand). In vitro, most of the compounds exhibited potent activity and the Ru derivatives **1a** [Ru(η^4-C_8H_{12})(2-Acpy-SMDT)Cl_2], **2a** [Ru(η^4-C_8H_{12})(2-Acpy-SBDT)Cl_2] and **3a** [Ru(η^4-C_8H_{12})(2-Acpy-TSC)Cl_2] were found more active than metronidazole against (HK-9) strain of *Entamoeba histolytica*.

Amoebiasis is a world-wide parasitic disease and a public health problem in developing countries,[1] which is responsible for up to 100,000 deaths per annum.[2] Metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole], is one of the most effective antiamoebic medications. However, it has mutagenic effect in bacteria, is carcinogenic to rodents[3] and is associated with transient myopia, neuropathy and immunosuppression.[4] There have been few reports of resistance of *E. histolytica* to metronidazole.[5] The drug is relatively ineffective against asymptomatic infection in the intestinal lumen.[3] Therefore, the ideal treatment for this disease does not exist and new agents are required.[6] There is rapidly growing interest in the use of transition metal complexes in medicine and other biological areas, particularly in the field of cancer.[7] Heterocyclic thiosemicarbazone and its metal complexes are important due to their biological activity.[8] Thiosemicarbazone derivatives are important due to their antibacterial,[9] antiprotozoal,[10] antiviral[11] and antineoplastic[12] activities. The Ru complexes were also tested against malaria[13] and trypanosoma.[14] The antitumor properties of a number of transition metal complexes of methyl 3-[1-(2-pyridyl) ethylidene] carbodithioate[15] have been reported. Earlier studies on Ru complexes, such as *cis*-$RuCl_2(DMSO)_4$ as antineoplastic agents, have suggested a DNA binding mechanism.[16] [$RuCl_2(DMSO)_2$(4-nitroimidazole)$_2$] has been used as radiosensitizer successfully.[16] 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 these ions when coordinated to nitrogen ligand.[17]

In a previous paper of this series we have described our strategy for the development of alternative metal based drugs against *E. histolytica*.[18] Continuing our efforts to develop improved antiamoebic drugs, we now report that coordination compounds of the following ligands (Fig. 1) with 1,5-cyclooctadiene ruthenium(II) fragment produce new compounds which are very active against in vitro culture of *E. histolytica*.

Chemistry

The precursor [Ru(η^4-C_8H_{12})$(CH_3CN)_2Cl_2$] was synthesized as reported by Albers et al.[19] *S*-Methyldithiocarbazate,[20] *S*-benzyldithiocarbazate[21] and 2-(2-pyridyl) benzimidazole[22] were prepared using literature procedures. The Schiff bases, 2-Acpy-SMDT (**1**), 2-Acpy-SBDT (**2**) and 2-Acpy-TSC (**3**) were prepared by refluxing equimolar amounts of 2-acetylpyridine and the respective amine in methanol. Their purities were checked by their melting point determinations and structures were confirmed by IR, ^{1}H NMR and electronic spectral studies. All (η^4-C_8H_{12})Ru(II) complexes were prepared by mixing an appropriate ligand (0.001 mol) and [Ru(η^4-C_8H_{12}) $(CH_3CN)_2Cl_2$] (0.001 mol) in refluxing methanol. The

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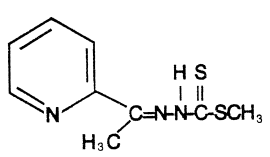
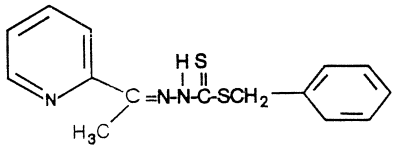


1
(2-Acpy-SMDT)



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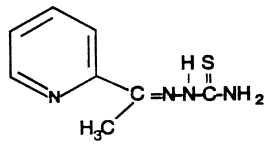
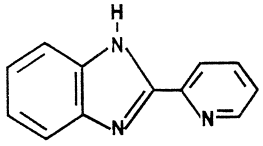
(2-Acpy-SBDT)



3
(2-Acpy-TSC)



4
2-(2-Py-BMZ)

Figure 1. 2-Acpy-SMDT = 2-acetylpyridine-*S*-methyldithiocarbazate, 2-Acpy-SBDT = 2-acetylpyridine-*S*-benzyldithiocarbazate, 2-Acpy-TSC = 2-acetylpyridine thiosemicarbazide, 2-(2-Py-BMZ) = 2-(2-pyridyl)benzimidazole.

product thus separated was filtered after cooling the solution at 0 °C overnight and washed with methanol.

$$[Ru(\eta^4\text{-}C_8H_{12})(CH_3CN)_2Cl_2] + L \xrightarrow[5\,h,\ reflux]{CH_3OH} [Ru(\eta^4\text{-}C_8H_{12})(L)Cl_2] + 2CH_3CN$$

(where L = ligands **1**, **2**, **3** and **4**).

Recrystallizations were effected in methanol:DMF (9:1). All these complexes are insoluble in water, sparingly soluble in methanol and ethanol, and fairly soluble in DMF and DMSO. These complexes are high melting solids and decompose before their melting temperature. Analytical and spectral data (IR, electronic and ^{1}H NMR)[23] are in good agreement with the composition $[Ru(\eta^4\text{-}C_8H_{12})(L)Cl_2]$. Analytical and other physicochemical data of the complexes are presented in Table 1. The band due to ν(C=N) (ring) of the pyridine moiety remains unaltered in **1a**, **2a** and **3a** (Fig. 2) while the shift of ν(C=N) (azomethine) to higher frequency in comparison to their respective free ligand, indicates the coordination of azomethine nitrogen to ruthenium. A strong band at 1050–1083 cm^{-1} ascribed to ν(C=S) of ligands is shifted to lower frequency (20–57 cm^{-1}), indicating the bonding of metal through thionic sulphur. The preferential coordination of sulphur over pyridinic nitrogen is due to the more nucleophilic character of the former. Thus, an octahedral structure (Fig. 2) is suggested for these complexes. Spectral data (IR as well as ^{1}H NMR) of **4a** are comparable to $[M_0O_2\{2\text{-}(2\text{-py-BMZ})\}]$ where coordination through both nitrogen atoms has been confirmed.[24]

Biological Properties

Preliminary experiments were carried out to determine the antiamoebic activities of ligands (**1**–**4**) and their complexes (**1a**–**4a**) using in vitro culture against HK-9 strain of *E. histolytica* as previously described[25] using a method in which amoeba were grown in a 96-well

Table 1. Analytical and physicochemical data of complexes

S. No.	Compound/stoichiometry[a]	Colour	Yield (%)	Dec. temp (°C)	Found (Calcd)			
					C	H	N	Cl
1a	$[Ru(\eta^4\text{-}C_8H_{12})(2\text{-Acpy-SMDT})Cl_2]$ $C_{17}H_{23}N_3S_2Cl_2Ru$	Steel gray	53	300	40.28 (40.39)	4.59 (4.55)	8.09 (8.32)	14.30 (14.06)
2a	$[Ru(\eta^4\text{-}C_8H_{12})(2\text{-Acpy-SBDT})Cl_2]$ $C_{23}H_{27}N_3S_2Cl_2Ru$	Dark brown	61	250	47.67 (47.50)	4.34 (4.65)	7.20 (7.23)	12.47 (12.22)
3a	$[Ru(\eta^4\text{-}C_8H_{12})(2\text{-Acpy-TSC})Cl_2]$ $C_{16}H_{22}N_4SCl_2Ru$	Yellow/cream	65	250	40.21 (40.50)	4.46 (4.64)	11.59 (11.81)	15.22 (14.98)
4a	$[Ru(\eta^4\text{-}C_8H_{12})\{2\text{-}(2\text{-Py-BMZ}\}Cl_2]$ $C_{20}H_{21}N_3Cl_2Ru$	Dark brown	54	235	50.31 (50.52)	4.80 (4.42)	9.01 (8.84)	14.62 (14.94)

[a]For abbreviations see Fig. 1.

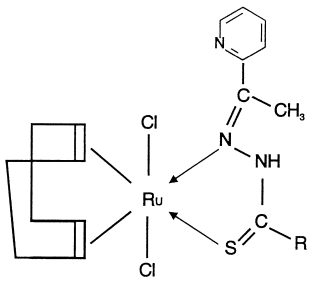


Figure 2. Structure of ruthenium complexes (**1a**: R = S-CH_3, **2a**: R = S-$CH_2C_6H_5$, **3a**: R = NH_2).

Table 2. Antiamoebic activity of ligands and their ruthenium complexes against HK-9 strain of *E. histolytica*

S. No.	Compound	IC_{50} μg/mL	S.D.[a]×10
1	2-Acpy-SMDT	0.38	0.28
1a	[Ru(η^4-C_8H_{12})(2-Acpy-SMDT)Cl_2]	0.28	0.42
2	2-Acpy-SBDT	0.40	0.14
2a	[Ru(η^4-C_8H_{12})(2-Acpy-SBDT)Cl_2]	0.26	0.35
3	2-Acpy-TSC	0.31	0.56
3a	[Ru(η^4-C_8H_{12})(2-Acpy-TSC)Cl_2]	0.30	0.28
4	2-(2-Py-BMZ)	0.42	0.42
4a	[Ru(η^4-C_8H_{12}){2-(2-Py-BMZ)}Cl_2]	0.36	0.42
5	Metronidazole	0.33	0.56

[a]S.D. = Standard deviation.

microplate. After 72 h incubation in the presence of serial dilutions of the compounds under test, inhibition of growth was assessed by fixing and then staining the organism with eosin and measuring the optical density with a microplate reader. Metronidazole was used as the reference drug. The biological test was carried out using DMSO as the solvent in which the complexes are stable. The in vitro antiamoebic activity of new ruthenium complexes and its ligands are listed in Table 2.

Metronidazole had a 50% inhibitory concentration (IC_{50}) of 0.33 μg/mL in our experiments which is close to the previously reported IC_{50} of 0.32 μg/mL obtained against *E. histolytica*.[26] As shown in Table 2, complexes **1a** and **2a** cause a marked inhibition, while the parent compounds are less active than metronidazole. Compounds **3a** and **4a** showed slightly better activity as compared to **3** and **4**. These activities indicate that the incorporation of the metal fragments generally produced an enhancement of the activity. Biological tests on the effect of complexes are very encouraging. This means that complexation to Ru increases the activity of the parental drug. These results illustrate well the potential of the novel metal-based approach for the development of chemotherapeutic agents against *E. histolytica*. Detailed in vivo studies, modification of these complexes and their mechanism of action are in progress.

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23. **1a** ^{1}H NMR (DMSO-d_6) δ 3.34 (m, 4H, CH), 2.84 (m, 4H, *exo* CH_2), 2.08 (m, 4H, *endo* CH_2), 2.54 (s, 3H, CH_3), 2.61 (s, 3H, SCH_3), 7.10–7.75 (m, 4H, aryl); IR *v* (cm^{-1}) 3200 (NH), 1641 (C=N), 1587 (C=C), 1032 (C=S), 510, 482, 450 (Ru–N, Ru–S); λ max (cm^{-1}) (DMF) 30300, 26315, 18180. **2a** ^{1}H NMR (DMSO-d_6) δ 3.50 (m, 4H, CH), 2.88 (m, 4H, *exo* CH_2), 7.20–7.98 (m, 9H, aryl); IR *v* (cm^{-1}) 3200 (NH), 1595 (C=N), 1575 (C=C), 1030 (C=S), 509, 472 (Ru–N, Ru–S); λ max (cm^{-1}) (DMF) 29410, 19010. **3a** ^{1}H NMR (DMSO-d_6) δ 2.50 (m, 4H, *exo* CH_2), 2.08 (m, 4H, *endo* CH_2), 2.87 (s, 3H, CH_3), 7.83–8.85 (m, 4H, aryl); IR *v* (cm^{-1}) 3250 (NH), 1613 (C=N), 1577 (C=C), 1026 (C=S), 526, 457, 420 (Ru–N); λ max (cm^{-1}) (DMF) 31847, 27777. **4a** ^{1}H NMR (DMSO-d_6) δ 3.58 (m, 4H, CH), 2.61 (m, 4H, *exo* CH_2), 2.26 (m, 4H, *endo* CH_2), 7.60–8.30 (m, 4H, aryl); IR *v* (cm^{-1}) 3050 (NH), 1613 (C=N), 1585 (C=C), 508, 454 (Ru–N); λ max (cm^{-1}) (DMF) 31950, 29410.
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