Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Medicinal Chemistry 41 (2006) 592–598
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://france.elsevier.com/direct/ejmech

Original article

Cyclooctadiene Ru(II) complexes of thiophene-2-carboxaldehyde-derived thiosemicarbazones: synthesis, characterization and antiamoebic activity

Shailendra Singh [a], Fareeda Athar [a], Mannar R. Maurya [b], Amir Azam [a,*]

[a] Department of Chemistry, Jamia Millia Islamia, Jamia Nagar, New Delhi 110025, India
[b] Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee 247667, India

Received 13 July 2005; revised and accepted 12 January 2006
Available online 20 March 2006

Abstract

Thiosemicarbazones (TSC) **1–10** were synthesized by condensing substituted thiosemicarbazide with thiophene-2-carboxaldehyde. These thiosemicarbazones were further reacted with $[Ru(\eta^4 -C_8H_{12})(CH_3CN)_2Cl_2]$ to form complexes of the type $[Ru(\eta^4 -C_8H_{12})(TSC)Cl_2]$ **1a–10a**. Thiosemicarbazones exhibited antiamoebic activity in the range IC_{50} = 1.09–5.42 μM. In vitro assessment of antiamoebic activity indicated that the thiosemicarbazones **3**, IC_{50} = 1.67 μM, **4**, IC_{50} = 1.11 μM and **6,** IC_{50} = 1.09 μM showed substantially less IC_{50} value than metronidazole (IC_{50} = 1.87 μM), a commonly used drug against amoebiasis. Cyclooctadiene Ru(II) complexes of thiosemicarbazones showed significant improvement in antiamoebic activity (IC_{50} = 0.30–1.39 μM). All the complexes possess noteworthy potencies and showed less IC_{50} values than metronidazole against *HK-9* strain of *Entamoeba histolytica*. Among all the complexes, the most promising antiamoebic activities was shown by the complexes **4a** and **6a** (IC_{50} = 0.31 μM of **4a** and IC_{50} = 0.30 μM of **6a** versus metronidazole).
© 2006 Elsevier SAS. All rights reserved.

Keywords: Thiophene-2-carboxaldehyde; Thiosemicarbazones; Ru(II) Complexes; Antiamoebic activity; *Entamoeba histolytica.*

1. Introduction

Amoebiasis, a protozoan infection, is the second leading cause of death. It is associated with liver and brain abscess and afflicts over 50 million people per annum leading to 50,000–100,000 deaths annually [1]. Recently, a case of invasive lung amoebiasis in which patient showed superior vena cava (SVC) syndrome and a brain abscess without liver involvement is reported. [2] The drugs currently used in the clinical treatment, nitroimidazole/metronidazole are considered to be the most effective against this parasitic disease but do not warrant complete cure. *Entamoeba histolytica*, the causative agent of amoebiasis, is able to adapt to therapeutically relevant levels of the drug and may develop resistance to nitroimidazoles/metronidazole [3]. The long-term use of medications produces undesirable side effects in patients [4]. Therefore, research on new drugs for the treatment still constitutes an important therapeutic demand. Thiosemicarbazones, a class of compounds, has been tested for their potential antitumor [5], antimalarial [6], antiviral activity [7], radio protector [8], anticonvulsant [9], trypanocidal agents [10–12], ulcer inhibitor and anticancer agents [13–15]. The thiosemicarbazones of thiophene-2-carboxaldehyde and their antileukemic activity are reported previously [16,17]. The significant leishmanicidal, anticancer and anti-HIV activity [18,19] of hydrazones of thiophene-2-carboxaldehyde led us to study the screening of thiosemicarbazones against *E. histolytica*.

$(ImH)[trans\text{-}Ru(Im)_2Cl_4]$ has been used as anticancer agent [20]. Earlier studies on Ru complexes such as, *cis*-$RuCl_2(DMSO)_4$, as antineoplastic agents, have suggested a DNA binding mechanism [21]. The complex $RuCl_2(CTZ)_2$ (CTZ = clotrimazole) displays good activity against *Trypanosoma cruzi*, [22] while $RuCl_2(CQ)_2$ (CQ = chloroquine) is efficient against *Plasmodium falciparum* [23]. In our earlier studies, some thiophene-2-carboxaldehyde thiosemicarbazones and Ru(II) and Pd(II) complexes showed promising results against *E. histolytica* [24,25]. In this paper we report the synthesis and characterization of new thiosemicarbazones of thio-

* Corresponding author. Tel.: +91 11 2698 1717x3253; fax: +91 11 2698 0229, +91 11 2698 1232.
E-mail address: amir_sumbal@yahoo.co.in (A. Azam).

0223-5234/$ - see front matter © 2006 Elsevier SAS. All rights reserved.
doi:10.1016/j.ejmech.2006.01.014

phene-2-carboxaldehyde and their ruthenium (II) complexes, in which the *N*-4 thiosemicarbazone moiety is replaced by cyclic and arylic amines. These compounds were tested in vitro for their ability to inhibit the growth of *E. histolytica* using micro-dilution method.

2. Experimental

Reactions were monitored by thin-layer chromatography (TLC) using Merck silica gel 60 F_{254} precoated thin layer plates. All the chemicals were purchased from Aldrich chemical company (USA). Elemental analyses (C, H, N) were carried out by Central Drug Research Institute, Lucknow, India, and the results were within 0.4% of the theoretical values. Chlorine was estimated by decomposing the complexes with Na_2O_2/NaOH and precipitated as AgCl with $AgNO_3$ after dissolving in dil. HNO_3. Melting points were recorded on a KSW melting point apparatus and were uncorrected. Electronic spectra were recorded in methanol on a Shimadzu UV-1601 PC UV-Visible spectrophotometer. IR spectra on KBr disks were recorded on a Perkin Elmer model 1620 FT-IR spectrophotometer. ^{1}H NMR data were obtained at ambient temperature using a Bruker Spectrospin DPX-300 MHz spectrophotometer in $CDCl_3$ for ligands and DMSO for complexes using tetramethylsilane as an internal standard. Splitting patterns are designated as follows; s, singlet; d, doublet; t, triplet; m, multiplet. Chemical shift values are given in (ppm). Thermograms of the complexes were recorded under nitrogen on a TG 51 thermogravimetric analyzer with increasing the temperature at 10 °C min^{-1}.

2.1. Synthesis of thiosemicarbazones: general method

All thiosemicarbazones were synthesized by mixing an aqueous solution of thiocarbonylhydrazines (0.003 mole in 10 ml) and ethanolic solution of thiophene-2-carboxaldehyde (0.003 mole in 10 ml) at 25 °C for 3 h with continuous stirring. After cooling, the precipitated compound was filtered and recrystallized from appropriate solvent.

2.1.1. 4-Cyclohexyl-4-methyl-1-(thiophene-2-ylmethylene) thiosemicarbazide, [2-TCA-NMCHTSC] (1)

Light brown solid (chloroform). Yield: 65%; m.p. 192 °C. *Anal*. calc. for $C_{13}H_{19}N_3S_2$: C 55.48, H 6.80, N 14.93; found: C 55.50, H 6.85, N 14.90%; UV–vis: ν_{max} (cm^{-1}) 29154, 38023, 47846; IR: ν_{max} (cm^{-1}) 3154 (NH), 1585 (C = N), 1497 (C = C), 1143 (C–N), 1076 (C = S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 9.32 (1H, s, –NH), 8.71 (1H, s, –CH = N), 7.26–7.98 (3H, m, aryl), 3.69 (1H, m, –CH), 3.57 (10H, m, –CH_2), 1.60 (3H, s, –CH_3).

2.1.2. 4-Methylpiperidine-1-(thiophene-2-ylmethylene) thiosemicarbazide, [2-TCA-4MPTSC] (2)

Yellow solid (methanol). Yield: 68%; m.p. 140 °C. *Anal*. calc. for $C_{12}H_{17}N_3S_2$: C 53.90, H 6.41, N 15.71; found: C 53.81, H 6.45, N 15.79%; UV–vis: ν_{max} (cm^{-1}) 30120, 37174, 49019; IR: ν_{max} (cm^{-1}) 3167 (NH), 1663 (C = N), 1493 (C = C), 1141 (C–N), 1067 (C = S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 9.07 (1H, s, –NH), 7.89 (1H, s, –CH = N), 7.20–7.59 (3H, m, aryl), 4.91 (1H, m, –CH), 4.79 (8H, m, –CH_2), 1.78 (3H, d, J = 5.3 Hz, –CH_3),.

2.1.3. 4-(2-Ethylpiperidine)-1-(thiophene-2-ylmethylene) thiosemicarbazide, [2-TCA-2EPTSC] (3)

Yellow solid (methanol/chloroform). Yield: 59%; m.p. 210 °C. *Anal.* calc. for $C_{13}H_{19}N_3S_2$: C 55.48, H 6.80, N 14.93; found: C 55.46, H 6.84, N 15.08%; UV–vis: ν_{max} (cm^{-1}) 28985, 37037, 48780; IR: ν_{max} (cm^{-1}) 3147 (NH), 1531 (C = N), 1492 (C = C), 1130 (C–N), 1045 (C = S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.81 (1H, s, –NH), 8.24 (1H, s, –CH = N), 7.09–7.98 (3H, m, aryl), 3.61 (1H, m, –CH), 3.27 (10H, m, –CH_2), 1.75 (3H, d, J = 5.1 Hz, –CH_3).

2.1.4. 4-(4-Benzylpiperidine)-1-(thiophene-2-ylmethylene) thiosemicarbazide, [2-TCA-4BPTSC] (4)

Yellow solid (methanol). Yield: 62%; m.p. 200 °C. *Anal*. calc. for $C_{18}H_{21}N_3S_2$: C 62.97, H 6.12, N 12.24; found: C 63.04, H 6.18, N 12.20%; UV–vis: ν_{max} (cm^{-1}) 29940, 37453, 47846; IR: ν_{max} (cm^{-1}) 3212 (NH), 1542 (C = N), 1499 (C = C), 1145 (C–N), 1072 (C = S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.99 (1H, s, –NH), 8.24 (1H, s, –CH = N), 7.09–7.49 (8H, m, aryl), 4.80 (1H, m, –CH), 4.75 (10H, m, –CH_2).

2.1.5. 4-(4-Phenylpiperazine)-1-(thiophene-2-ylmethylene) thiosemicarbazide, [2-TCA-NPPTSC] (5)

Light brown solid (methanol). Yield: 63%; m.p. 115 °C. *Anal*. calc. for $C_{16}H_{18}N_4S_2$: C 58.15, H 5.49, N 16.95; found: C 58.13, H 5.52, N 17.05%; UV–vis: ν_{max} (cm^{-1}) 29154, 37843, 48309; IR: ν_{max} (cm^{-1}) 3120 (NH), 1600 (C = N), 1545 (C = C), 1132 (C–N), 1018 (C = S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 10.37 (1H, s, –NH), 8.54 (1H, s, –CH = N), 6.87-7.85 (8H, m, aryl), 4.21 (8H, m, –CH_2).

2.1.6. 4-(2-Adamantyl)-1-(thiophene-2-ylmethylene) thiosemicarbazide, [2-TCA-ADMTSC] (6)

White solid (ethanol/acetone). Yield: 57%; m.p. 115 °C. *Anal*. calc. for $C_{16}H_{22}N_3S_2$: C 59.96, H 6.92, N 13.11; found: C 60.09, H 7.06, N 13.09%; UV–vis: ν_{max} (cm^{-1}) 29154, 38167, 48076; IR: ν_{max} (cm^{-1}) 3200 (NH), 1650 (C = N), 1535 (C = C), 1135 (C–N), 1057 (C = S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 9.67 (1H, s, –NH), 8.45 (1H, d, –NH), 7.69 (1H, s, –CH = N), 7.02–7.45 (3H, m, aryl), 4.05–4.13 (18H, m, adamantyl ring).

2.1.7. 4-o-Toluidine-1-(thiophene-2-ylmethylene) thiosemicarbazide, [2-TCA-o-TolTSC] (7)

White solid (acetone). Yield: 47%; m.p. 205 °C. *Anal*. calc. for $C_{13}H_{13}N_3S_2$: C 56.70, H 4.76, N 15.26; found: C 56.75, H 4.80, N 15.30%; UV–vis: ν_{max} (cm^{-1}) 29850, 37526, 47393; IR: ν_{max} (cm^{-1}) 3215 (NH), 1548 (C = N), 1490 (C = C), 1107 (C–N), 1040 (C = S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 9.97 (2H, s, –NH), 8.87 (1H, s, –CH = N), 7.65–8.11 (7H, m, aryl), 2.30 (3H, s, –CH_3).

*2.1.8. 4-m-Toluidine-1-(thiophene-2-ylmethylene) thiosemicarbazide, [2-TCA-m-TolTSC] (**8**)*

White solid (acetone). Yield: 45%; m.p. 196 °C. *Anal*. calc. for $C_{13}H_{13}N_3S_2$: C 56.70, H 4.76, N 15.26; found: C 56.75, H 4.81, N 15.30%; UV–vis: ν_{max} (cm^{-1}) 29585, 38759, 47393; IR: ν_{max} (cm^{-1}) 3168 (NH), 1556 (C = N), 1511 (C = C), 1119 (C–N), 1041 (C = S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 9.71 (2H, s, –NH), 8.24 (1H, s, –CH = N), 7.18–7.49 (7H, m, aryl), 2.35 (3H, s, $–CH_3$).

*2.1.9. 4-p-Toluidine-1-(thiophene-2-ylmethylene) thiosemicarbazide, [2-TCA-p-TolTSC] (**9**)*

Light yellow solid (methanol). Yield: 54%; m.p. 192 °C. *Anal*. calc. for $C_{13}H_{13}N_3S_2$: C 56.70, H 4.76, N 15.26; found: C 56.71, H 4.82, N 15.31%; UV–vis: ν_{max} (cm^{-1}) 29761, 38167, 48309; IR: ν_{max} (cm^{-1}) 3260 (NH), 1537 (C = N), 1492 (C = C), 1163 (C–N), 1087 (C = S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 9.53 (2H, s, –NH), 8.80 (1H, s, –CH = N), 6.96–7.33 (7H, m, aryl), 2.29 (3H, s, $–CH_3$).

*2.1.10. 4-(4-Methylbenzyl)-1-(thiophene-2-ylmethylene) thiosemicarbazide, [2-TCA-NMBzlTSC] (**10**)*

Yellow solid (methanol/chloroform). Yield: 56%; m.p. 195 °C. *Anal*. calc. for $C_{14}H_{15}N_3S_2$: C 58.10, H 5.22, N 14.52; found: C 58.05, H 5.24, N 14.61%; UV–vis: ν_{max} (cm^{-1}) 29498, 37598, 47619; IR: ν_{max} (cm^{-1}) 3170 (NH), 1598 (C = N), 1515 (C = C), 1147 (C–N), 1088 (C = S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 9.17 (1H, s, –NH), 7.89 (1H, s, –CH = N), 6.96–7.61 (8H, m, aryl), 3.42 (2H, s, $–CH_2$), 1.79 (3H, s, $–CH_3$).

2.2. Preparation of complexes: general method

A solution of appropriate thiosemicarbazone (0.001 mole) in dry methanol (10 ml) was added to a stirred suspension of $[Ru(\eta^4\text{-}C_8H_{12})\ (CH_3CN)_2Cl_2]$ (0.001 mole) in hot methanol (10 ml). The obtained mixture was heated at reflux on a water bath for 4 h during which period starting material dissolved and complex started to separate. After keeping the reaction flask at room temperature for 2 h, the brown solid was filtered, washed with methanol and dried in vacuo over silica gel.

*2.2.1. Dichloro (4-cyclohexyl-4-methyl-1-(thiophene-2-ylmethylene)thiosemicarbazide) cyclooctadiene ruthenium(II) [Ru(η^4-C_8H_{12})(2-TCA-NMCHTSC)Cl_2] (**1a**)*

Brown solid (methanol/DMSO); yield: 54%; dec. temp. 207 °C. *Anal*. calc. for $C_{21}H_{31}N_3S_2Cl_2Ru$: C 44.91, H 5.56, N 7.48, Cl 12.63; found: C 45.07, H 5.60, N 7.40, Cl 12.71%; UV–vis: ν (cm^{-1}) 22010, 36736, 49419; IR: ν_{max} (cm^{-1}) 3237 (NH), 1607 (C = N), 1484 (C = C), 1057 (C = S), 514, 578, 541 (Ru-N, Ru-S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 8.69 (1H, s, –CH = N), 6.94–7.18 (3H, m, aryl), 4.98 (1H, s, –NH), 3.57 (1H, m, –CH), 3.21 (3H, s, $–CH_3$), 2.92 (10H, s, $–CH_2$), 2.54 (4H, m, *exo* CH_2), 2.18 (4H, m, *endo* CH_2).

*2.2.2. Dichloro (4-methylpiperidine-1-(thiophene-2-ylmethylene)thiosemicarbazide) cyclooctadiene ruthenium(II) [Ru(η^4-C_8H_{12})(2-TCA-4MPTSC)Cl_2] (**2a**)*

Dark brown solid (methanol/DMSO); yield: 71%; dec. temp. 271 °C. *Anal*. calc. for $C_{20}H_{29}N_3S_2Cl_2Ru$: C 43.87, H 5.34, N 7.67, Cl 12.95; found: C 43.90, H 5.39, N 7.70, Cl 12.90%; UV–vis: ν_{max} (cm^{-1}) 22331, 27548, 36995, 49816; IR: ν_{max} (cm^{-1}) 3284 (NH), 1591 (C = N), 1497 (C = C), 1039 (C = S), 508, 470, 438 (Ru-N, Ru-S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 7.89 (1H, s, –CH = N), 7.13–7.69 (3H, m, aryl), 4.70 (1H, m, –CH), 4.39 (8H, m, $–CH_2$), 3.49 (1H, s, –NH), 2.45 (3H, d, $–CH_3$), 2.39 (4H, m, *exo* CH_2), 2.01 (4H, m, *endo* CH_2).

*2.2.3. Dichloro (4-(2-ethylpiperidine)-1-(thiophene-2-ylmethylene)thiosemicarbazide) cyclooctadiene ruthenium(II) [Ru(η^4-C_8H_{12})(2-TCA-2EPTSC)Cl_2] (**3a**)*

Dark brown solid (methanol/DMSO); yield: 65%; dec. temp. 286 °C. *Anal*. calc. for $C_{21}H_{31}N_3S_2Cl_2Ru$: C 44.91, H 5.56, N 7.48, Cl 12.63; found: C 44.95, H 5.60, N 7.50, Cl 12.57%; UV–vis: ν_{max} (cm^{-1}) 21945, 26316, 37878, 49160; IR: ν_{max} (cm^{-1}) 3435 (NH), 1561 (C = N), 1509 (C = C), 1018 (C = S), 489, 456 (Ru-N, Ru-S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 8.54 (1H, s, –CH = N), 7.07–7.39 (3H, m, aryl), 4.95 (1H, s, –NH), 3.58 (1H, m, –CH), 3.31 (10H, m, $–CH_2$), 2.65 (4H, m, *exo* CH_2), 2.39 (4H, m, *endo* CH_2), 1.95 (3H, t, $–CH_3$).

*2.2.4. Dichloro (4-(4-benzylpiperidine)-1-(thiophene-2-ylmethylene)thiosemicarbazide) cyclooctadiene ruthenium(II) [Ru(η^4-C_8H_{12})(2-TCA-4BPTSC)Cl_2] (**4a**)*

Brown solid (methanol/DMSO); yield: 69%; dec. temp. 279 °C. *Anal*. calc. for $C_{26}H_{33}N_3S_2Cl_2Ru$: C 50.07, H 5.33, N 6.74, Cl 11.37; found: C 49.95, H 5.39, N 6.80, Cl 11.40%; UV–vis: ν_{max} (cm^{-1}) 21890, 36736, 49508; IR: ν_{max} (cm^{-1}) 3437 (NH), 1553 (C = N), 1485 (C = C), 1056 (C = S), 519, 487, 442 (Ru-N, Ru-S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 8.61 (1H, s, –CH = N), 7.11–7.38 (8H, m, aryl), 4.95 (1H, s, –NH), 3.61 (1H, m, –CH), 3.10 (10H, m, $–CH_2$), 2.60 (4H, m, *exo* CH_2), 2.38 (4H, m, *endo* CH_2).

*2.2.5. Dichloro (4-(4-phenylpiperazine)-1-(thiophene-2-ylmethylene)thiosemicarbazide) cyclooctadiene ruthenium(II) [Ru(η^4-C_8H_{12})(2-TCA-NPPTSC)Cl_2] (**5a**)*

Dark brown solid (methanol/DMSO); yield: 72%; dec. temp. 285 °C. *Anal*. calc. for $C_{24}H_{30}N_4S_2Cl_2Ru$: C 47.21, H 4.95, N 9.18, Cl 11.61; found: C 47.25, H 5.00, N 9.22, Cl 11.66%; UV–vis: ν_{max} (cm^{-1}) 21431, 24938, 36995, 49513; IR: ν_{max} (cm^{-1}) 3256 (NH), 1629 (C = N), 1538 (C = C), 1012 (C = S), 497, 452 (Ru-N, Ru-S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 8.14 (1H, s, –CH = N), 7.13–7.61 (8H, m, aryl), 4.19 (1H, s, –NH), 3.92 (8H, s, $–CH_2$), 2.65 (4H, m, *exo* CH_2), 2.19 (4H, m, *endo* CH_2).

*2.2.6. Dichloro (4-(2-adamantyl)-1-(thiophene-2-ylmethylene) thiosemicarbazide) cyclooctadiene ruthenium(II) [Ru(η^4-C_8H_{12})(2-TCA-ADMTSC)Cl_2] (**6a**)*

Brown solid (methanol/DMSO); yield: 54%; dec. temp. 292 °C. *Anal*. calc. for $C_{24}H_{33}N_3S_2Cl_2Ru$: C 48.07, H 5.55,

N 7.01, Cl 11.82; found: C 47.97, H 5.59, N 7.10, Cl 11.79%; UV–vis: ν_{max} (cm^{-1}) 21998, 36315, 49513; IR: ν_{max} (cm^{-1}) 3281 (NH), 1669 (C = N), 1528 (C = C), 1027 (C = S), 511, 478, 437 (Ru-N, Ru-S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 8.03 (1H, s, –CH = N), 7.13–7.49 (3H, m, aryl), 3.83 (1H, s, –NH), 3.69–3.78 (18H, m, adamantyl ring), 2.53 (4H, m, *exo* CH_2), 2.34 (4H, m, *endo* CH_2).

*2.2.7. Dichloro (4-o-toluidine-1-(thiophene-2-ylmethylene) thiosemicarbazide) cyclooctadiene ruthenium(II) [Ru(η^4-C_8H_{12})(2-TCA-o-TolTSC)Cl_2] (**7a**)*

Dark brown solid (methanol/DMSO); yield: 74%; dec. temp. 241 °C. *Anal*. calc. for $C_{21}H_{25}N_3S_2Cl_2Ru$: C 45.40, H 4.54, N 7.56, Cl 12.76; found: C 45.45, H 4.60, N 7.60, Cl 12.77%; UV–vis: ν_{max} (cm^{-1}) 21946, 24630, 37736, 49915; IR: ν_{max} (cm^{-1}) 3355 (NH), 1557 (C = N), 1489 (C = C), 1070 (C = S), 509, 478, 439 (Ru-N, Ru-S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 8.10 (1H, s, –CH = N), 7.23–7.69 (7H, m, aryl), 3.93 (1H, s, –NH), 2.59 (4H, m, *exo* CH_2), 2.19 (4H, m, *endo* CH_2), 2.04 (3H, s, $–CH_3$).

*2.2.8. Dichloro (4-m-toluidine-1-(thiophene-2-ylmethylene) thiosemicarbazide) cyclooctadiene ruthenium(II) [Ru(η^4-C_8H_{12})(2-TCA-m-TolTSC)Cl_2] (**8a**)*

Brown solid (methanol/DMSO); yield: 68%; dec. temp. 235 °C. *Anal*. calc. for $C_{21}H_{25}N_3S_2Cl_2Ru$: C 45.40, H 4.54, N 7.56, Cl 12.79; found: C 45.32, H 4.61, N 7.60, Cl 12.80%; UV–vis: ν_{max} (cm^{-1}) 22127, 25915, 36418, 49423; IR: ν_{max} (cm^{-1}) 3295 (NH), 1581 (C = N), 1502 (C = C), 1054 (C = S), 484, 445 (Ru-N, Ru-S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 7.93 (1H, s, –CH = N), 7.10–7.38 (7H, m, aryl), 4.20 (1H, s, –NH), 2.61 (4H, m, *exo* CH_2), 2.24 (4H, m, *endo* CH_2), 2.01 (3H, s, $–CH_3$).

*2.2.9. Dichloro (4-p-toluidine-1-(thiophene-2-ylmethylene) thiosemicarbazide) cyclooctadiene ruthenium(II) [Ru(η^4-C_8H_{12})(2-TCA-p-TolTSC)Cl_2] (**9a**)*

Dark brown solid (methanol/DMSO); yield: 65%; dec. temp. 250 °C. *Anal*. calc. for $C_{21}H_{25}N_3S_2Cl_2Ru$: C 45.40, H 4.54, N 7.56, Cl 12.79; found: C 45.45, H 4.59, N 7.50, Cl 12.80%; UV–vis: ν_{max} (cm^{-1}) 21929, 28985, 37593, 49216; IR: ν_{max} (cm^{-1}) 3305 (NH), 1594 (C=N), 1515 (C=C), 1019 (C = S), 496, 458 (Ru-N, Ru-S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 7.89 (1H, s, –CH = N), 7.01–7.25 (7H, m, aryl), 3.99 (1H, s, –NH), 2.43 (4H, m, *exo* CH_2), 2.13 (4H, m, *endo* CH_2), 1.98 (3H, s, $–CH_3$).

*2.2.10. Dichloro (4-(4-methylbenzyl)-1-(thiophene-2-ylmethylene)thiosemicarbazide) cyclooctadiene ruthenium(II) [Ru(η^4-C_8H_{12})(2-TCA-NMBzlTSC)Cl_2] (**10a**)*

Dark brown solid (methanol/DMSO); yield: 66%; dec. temp. 298 °C. *Anal*. calc. for $C_{22}H_{27}N_3S_2Cl_2Ru$: C 46.39, H 4.78, N 7.38, Cl 12.45; found: C 46.41, H 4.79, N 7.40, Cl 12.50%; UV–vis: ν_{max} (cm^{-1}) 21445, 25773, 37154, 49152; IR: ν_{max} (cm^{-1}) 3254 (NH), 1621 (C = N), 1509 (C = C), 1057 (C = S), 516, 479, 438 (Ru-N, Ru-S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 8.72 (1H, s, –CH = N), 7.03–7.18 (8H, m, aryl), 4.21 (1H, s, –NH), 3.44 (2H, s, $–CH_2$), 3.31 (3H, s, $–CH_3$), 2.71 (4H, m, *exo* CH_2), 2.22 (4H, m, *endo* CH_2).

2.3. Organism culture and in vitro testing against E. histolytica

Preliminary experiments were carried out to determine the antiamoebic activities of the in vitro culture against the *HK-9* strain of *E. histolytica* as previously described [26]. The *E. histolytica* strain HK-9 was cultured in tubes (15 cm^3) by using Diamond TYIS-33 medium [27]. All the compounds were dissolved in DMSO (40 μl) [28,29] followed by adding enough culture medium to obtain concentration of 1 mg ml^{-1}. Stock solutions of the compounds were prepared freshly before use at a concentration of 0.1 mg ml^{-1}. Twofold serial dilutions were made in the wells of 96-well microtiter plate (Costar). Each test includes metronidazole as a standard amoebicidal drug, with control wells (culture medium plus amoebae) and a blank (culture medium only). All the experiments were carried out in triplicate at each concentration level and repeated thrice. The amoebae suspension was prepared from a confluent culture by pouring off the medium at 37 °C and adding 5 ml of fresh medium, chilling the culture tube on ice to detach the organisms from the side of the flask. The number of amoeba per ml was estimated with a haemocytometer, using trypan blue exclusion to confirm the viability. The suspension was diluted to 10^5 organism per ml by adding fresh medium and 170 μl of this suspension was added to the test and control wells in the plate so that the wells were completely filled (total volume, 340 μl). An inoculum of 1. 7×10^4 organisms per well was chosen so that confluent, but not excessive growth, took place in control wells. Plates were sealed and gassed for 10 min with nitrogen before incubation at 37 °C for 72 h. After incubation, the growth of amoebae in the plate was checked with a low power microscope. By inverting the plate, the culture media was removed with gentle shaking and then immediately washed with sodium chloride solution (0.9%) at 37 °C. This procedure was completed quickly and the plate was not allowed to cool in order to prevent the detachment of amoebae. It was allowed to dry at room temperature. After drying amoeba were fixed with chilled methanol by keeping it in ice bath for 15 min. dried and stained with (0.5%) aqueous eosin for 15 min. The stained plate was washed once with tap water, then twice with distilled water and allowed to dry. A 200 μl portion of 0.1 N sodium hydroxide solution was added to each well to dissolve the protein and release the dye. The optical density of the resulting solution in each well was determined at 490 nm with a microplate reader. The % inhibition of amoebal growth was calculated from the optical densities of the control and test wells and plotted against the logarithm of the dose of the drug tested. Linear regression analysis was used to determine the best fitting straight line from which the IC_{50} value was found.

3. Results and discussion

The thiosemicarbazone derivatives were synthesized using the method as reported earlier [30]. These thiosemicarbazones

are insoluble in water and highly soluble in chloroform, methanol, DMF and DMSO. The precursor $[Ru(\eta^4-C_8H_{12})(CH_3CN)_2Cl_2]$ used for the synthesis of Ru(II) complexes was synthesized by the literature procedure [31]. All compounds were characterized by elemental analysis, IR, ^1H NMR and electronic spectra and their purity was established by TLC and melting point. Analytical and spectral data are in good agreement with the composition of thiosemicarbazones **1-10** (Fig. 1) and their 1,5-cyclooctadiene Ru(II) complexes **1a**–**10a** (Fig. 2).

3.1. IR and electronic spectral studies

The IR spectra of the compounds are quite similar despite the presence of different substituent, which shows that the charge delocalization was not influenced by substituting different amines. All the thiosemicarbazones **1–10** may exhibit thione–thiol tautomerism, since they have a thione group (C = S) and proton adjacent to the thione group [32]. It has been stated that the thione group (C = S) is relatively unstable in the monomeric form and tends to turn into a stable C–S single bond by tautomerism, if there is at least one hydrogen atom adjacent to the C = S bond. However, all the compounds showed intense, strong bands in the region 1018–1092 cm^{-1} due to ν(C = S) stretch and no band near 2570 cm^{-1} due to ν(C–SH), suggesting that these ligands remain in the thione form in the solid-state. The highest frequency band observed in the 3147–3343 cm^{-1} region, due to the amine group stretching vibration in ligands also remains unaffected in the complexes. The downward (C = S) band shift (16–37 cm^{-1}) in the complexes suggested the coordination of thiocarbonyl sulfur. The spectra of all the thiosemicarbazones exhibit a strong band at 1531–1653 cm^{-1} region due to the ν(C = N) stretch of the azomethine linkage. In the complexes this band shifted (14–46 cm^{-1}) to lower frequency. This lowering of the ν(C = N) stretch on complexation may be attributed to lowering of the C = N bond order as a result of the M-N band formation. Bands in the region ~ 450 cm^{-1} are assigned to the ν(M-N) band which further supports the coordination of the azomethine nitrogen. The two strong bands observed at 716–680 and 605–597 cm^{-1} regions are attributed to the thiophene ring deformation modes in the ligands. These thiophene ring deformation vibrations are not affected in the complexes.

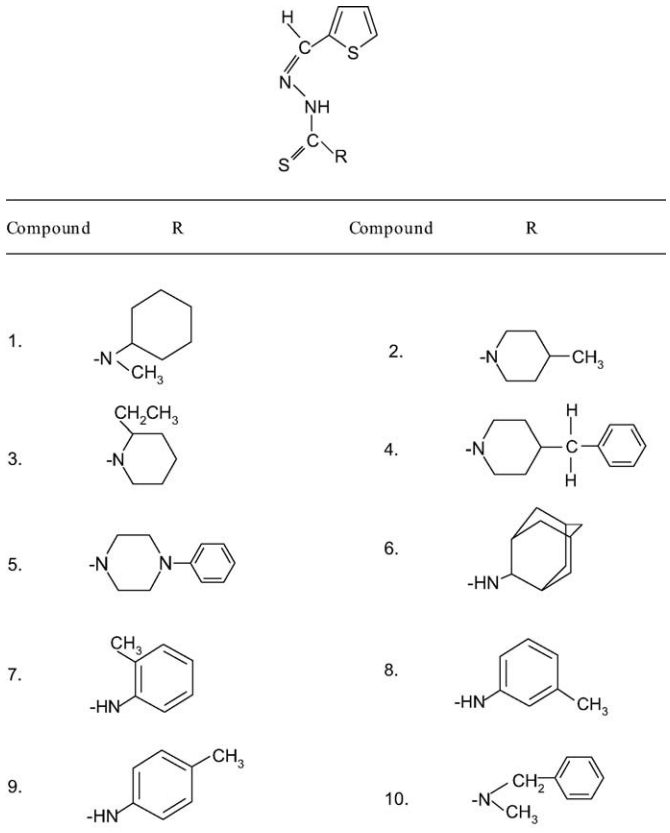

Fig. 1. Structure of the thiophene-2-carboxaldehyde thiosemicarbazones (**1**–**10**).

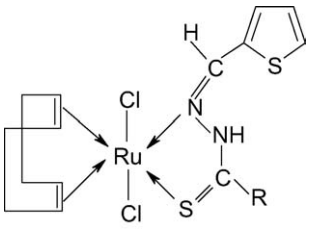

Fig. 2. General structure of the thiophene-2-carboxaldehyde thiosemicarbazone Ru(II) complexes (**1a**–**10a**).

The electronic spectra of the ligands exhibit three bands in the region 28,985–30,395, 37,037–38,759 and 47,393–48,880 cm^{-1}. The probable assignment for the first two bands is due to the *n* π* and while last one is due to π π* transitions. The thiosemicarbazones and their complexes exhibits π π* band at ca. 48,000 cm^{-1} and *n* π* band at ca. 37,000 cm^{-1}, with little change in the energy of these bands. In complexes n π* band was located at ca. 25,000 cm^{-1}. In addition, a charge transfer band was located in the lower energy region. For some complexes the two bands, which are intraligand, merge to form one band while in others the lower energy intraligand bands merge with charge transfer band.

3.2. ^1H NMR spectral studies

All the thiosemicarbazones exhibit signal due to the –NH proton in the region 8.81–11.39 ppm. This signal usually shifts to upfield and appears at 3.05–4.95 ppm after complexation. However, in some complexes, –NH proton signal could not be located in the 0–15 ppm range. This either merges with aromatic protons or resonate beyond 15 ppm. All other protons and aromatic protons resonate nearly at the same region with slight shielding to that of the corresponding free thiosemicarbazone.

3.3. Thermal studies (TGA)

The thermograms of the complexes along with the % loss at different temperatures were recorded under nitrogen. The T.G. profiles showed the complexes to be non-solvated and stable

up to 200 °C. Further increment of temperature caused decomposition of all the complexes. In all the decomposition steps loss of mixed fragments occurred which made it difficult to predict the loss of any particular group at any step. Although decomposed fragments of the ligands could not be approximated due to continuous weight loss of the respective ligand. The residue corresponds to the ruthenium sulfide, i.e. after considering the transfer of one sulfur atom to the metal ion.

3.4. Biological activity

The new thiosemicarbazones and their ruthenium(II) complexes were screened in vitro against HK-9 strain of *E. histolytica* for antiamoebic activity and the results are listed in Table 1. Metronidazole was used as a reference amoebicidal drug. The biological test was carried out using DMSO as the solvent in which all the compounds are stable. Metronidazole had an inhibitory concentration (IC_{50}) of 1.87 μM in our experiment, which is close to the previously reported IC_{50} of 1.81 μM obtained against same strain of *E. histolytica* [33]. The results were estimated as the percentage of growth inhibition compared with the untreated controls and plotted as probit values as a function of the drug concentration. The IC_{50} and 95% confidence limits were interpolated in the corresponding dose-response curve. The thiosemicarbazones **1–10** exhibit IC_{50} values in the range (1.09–5.42 μM). The ligands (**3**, IC_{50} = 1.67 μM, **4**, IC_{50} = 1.11 μM and **6,** IC_{50} = 1.09 μM) showed better amoebicidal activity as compared to metronidazole (IC_{50} = 1.87 μM). It was noted that antiparasitic activity was limited to those compounds in which the alkylidene group is attached to the 2- position, rather than 3- or 4- position of the heterocyclic ring and also to those in which a thiocarbonyl, rather than a carbonyl group, is present [34]. All the complexes **1a–10a** (0.30–1.39 μM) showed less IC_{50} value than metronidazole. The cyclic analogues of thiosemicarbazones were found to be more active than the arylic analogues. The most promising among them are Ru(II) complexes **4a** and **6a** having 4-benzylpiperidine and 2-adamantyl as N^4 substitution. The results were statistically evaluated by analysis of variance. The null hypothesis was tested using *t*-test. The significativity of the difference between the IC_{50} values of metronidazole and the complexes **1a–10a** was evaluated by *t*-test. The values of the calculated *T* were found higher than the Table value of *T* at 5% level, thus concluding that the character under study is said to be significantly influenced by the treatment. The Ru-complex precursor $[RuCl_2(\eta^4\text{-}C_8H_{12})(MeCN)_2]$ was also evaluated for antiamoebic activity. The IC_{50} value of the metal precursor establishes that it does not have any effect on growth of *E. histolytica*. The results indicate that the complexation to Ru increases the activity of parent ligands. Detailed studies of the toxicity of these compounds, mechanism of action as well as in vivo studies are in progress.

Table 1
In vitro antiamoebic activity of thiophene-2-carboxaldehyde thiosemicarbazones and their Ru(II) complexes against (*HK-9*) strain of *E. histolytica*

Compound	IC50 (μM)	S.D.
1	4.41	0.71
1a	1.23	0.13
2	2.58	0.18
2a	0.75	0.05
3	1.67	0.13
3a	0.52	0.03
4	1.11	0.07
4a	0.31	0.02
5	5.42	0.77
5a	1.39	0.15
6	1.09	0.13
6a	0.30	0.06
7	2.87	0.18
7a	0.81	0.12
8	3.13	0.35
8a	0.69	0.11
9	3.02	0.27
9a	0.74	0.09
10	2.56	0.31
10a	0.76	0.13
[RuCl2(η4-C8H12)(MeCN)2]	14.36	3.47
Metronidazole	1.87	0.47

4. Conclusion

In conclusion, thiosemicarbazones derivatives and their new Ruthenium(II) complexes derived from thiophene-2-carboxaldehyde represent an interesting new family of compounds to control growth of *E. histolytica*. The biological behavior revealed that the thiosemicarbazones **3**, **4** and **6** containing 2-ethylpiperidine, 4-benzylpiperidine and adamantylamino as N^4 substitution showed better IC_{50} value than metronidazole against *E. histolytica*. The chelation induced significant changes in the biological activity of the ligands. Though all the ruthenium complexes **1a–10a** have shown less IC_{50} value than metronidazole, the better antiamoebic activity was shown by cyclic analogues than arylic analogues. The most promising among them are the Ru(II) complexes **4a** and **6a** having 4-benzylpiperidine and adamantylamino as N^4 substitution.

Acknowledgements

This work was supported from Council of Scientific and Industrial Research (Grant no. 27(0117)/02/EMR-ll), New Delhi, India.

References

[1] H. Schuster, P.L. Chiodini, Curr. Opin. Infect. Dis. 14 (2001) 587–591.
[2] A. Lichtenstein, A.T. Kondo, G.S. Visvesvara, A. Fernandez, E.F. Paiva, T. Mauad, M. Dolhnikoff, M.A. Martins, Thorax 60 (2005) 350–352.
[3] I.S. Adagu, D. Nolder, D.C. Warhurst, J.F. Rossignol, J. Antimicrob. Chemother. 49 (2002) 103–111.
[4] C.E. Voogd, S.J.J. Vander, J.J. Jacobs, Mutat. Res. 26 (1974) 483–490.
[5] J. Easmon, G. Purstinger, G. Heinisch, T. Roth, H.H. Fiebig, W. Holzer, W. Jager, M. Jenney, J. Hofmann, J. Med. Chem. 44 (2001) 2164–2171.
[6] W. Seebacher, R. Brun, R. Weis, Eur. J. Pharm. Sci. 21 (2004) 225–233.
[7] A. Kolocouris, K. Dimas, C. Pannecouque, M. Witvrouw, G.B. Foscolos, G. Stamatiou, G. Fytas, G. Zoidis, N. Kolocouris, G. Andrei, R. Snoeck, E.D. Clercq, Bioorg. Med. Chem. Lett. 12 (2002) 723–727.

[8] M. Tarua, C. Ribuot, M.H. Pera, G. Taillandier, M. Fatome, J.D. Laval, P. Demenge, G. Leclerc, Eur. J. Med. Chem. 31 (1996) 589–595.
[9] J.R. Dimmock, S.N. Pandeya, J.W. Quail, U. Pugazhenthi, T.M. Allen, G.Y. Kao, J. Balzarini, E. Declercq, Eur. J. Med. Chem. 30 (1995) 303–314.
[10] G. Aguirre, L. Boiani, H. Cerecetto, M. Fernández, M. González, A. Denicola, L. Otero, D. Gambino, C. Rigol, C. Olea-Azar, M. Faundez, Bioorg. Med. Chem. 12 (2004) 4885–4893.
[11] X. Du, C. Guo, E. Hansell, P.S. Doyle, C.R. Caffrey, T.P. Holler, J.H. McKerrow, F.E. Cohen, J. Med. Chem. 45 (2002) 2695–2707.
[12] X. Du, E. Hansell, J.C. Engel, C.R. Caffrey, F.E. Cohen, J.H. McKerrow, Chem. Biol. 7 (2000) 733–742.
[13] I.H. Hall, O.T. Wong, J.M. Chapman, Anticancer Drugs 6 (1995) 147.
[14] M.C. Liu, T.S. Lin, A.C. Sartorelli, Prog. Med. Chem. 32 (1995) 1–35.
[15] M.C. Liu, T.S. Lin, J.G. Cory, A.H. Cory, J. Med. Chem. 39 (1996) 2586–2593.
[16] J. Garcia-Tojal, A. Garcia-Orad, J.L. Serra, J.L. Pizarro, L. Lezama, M.I. Arriortua, T. Rojo, J. Inorg. Biochem. 75 (1999) 45–54.
[17] J. Garcia-Tojal, J.L. Pizarro, A. Garcia-Orad, A.R. Perez-Sanz, M.M. Ugalde, A.A. Dýaz, J.L. Serra, M.I. Arriortua, T. Rojo, J. Inorg. Biochem. 86 (2001) 627–633.
[18] B. Savornin, N.E. Madadi, F. Delmas, M. Gasquit, P. Timon-David, P. Vanelle, P., J. Meldonado, J. Pharm. Pharmacol. 43 (1991) 58–59.
[19] N. Ocal, S. Kaban, Ind. J. Chem. 37B (1999) 1051–1053.
[20] F. Kratz, M. Hartmann, B. Keppler, R. Messori, J. Biol. Chem. 269 (1994) 2581.
[21] N. Farrel, in: S.J. Lippard (Ed.), In Chemistry and Biochemistry of Platinum, Gold and other Chemotherapeutic Agents, ACS Symp, 1983, pp. 279–297 (209).
[22] R.A. Sanchez-Delgado, K. Lazardi, L. Rincon, J.A. Urbina, A.J. Hurbert, A.N. Noels, J. Med. Chem. 36 (1993) 2041–2043.
[23] R.A. Sanchez-Delgado, M. Navarro, H. Perez, J.A. Urbina, J. Med. Chem. 39 (1996) 1095–1099.
[24] N. Shailendra, M.T.G. Bharti, D.E. Garza, J.C. Cruz-Vega, K. Garza, F. Saleem, A. Naqvi, Azam, Bioorg. Med. Chem. Lett. 11 (2001) 2675–2678.
[25] N. Shailendra, F. Bharti, A. Naqvi, Azam, Bioorg. Med. Chem. Lett. 13 (2003) 689–692.
[26] C.W. Wright, M.J. O'Neill, J.D. Phillipson, D.C. Warhurst, Antimicrob. Agents Chemother. 32 (1988) 1725–1729.
[27] L.S. Diamond, D.R. Harlow, C.C. Cunnick, C.C. Trans, R. Soc. Trop. Med. Hyg. 72 (1978) 431–432.
[28] F.D. Gillin, D.S. Reiner, M. Suffness, Antimicrob. Agents Chemother. 22 (1982) 342–345.
[29] A.T. Keene, A. Harris, J.D. Phillipson, D.C. Warhurst, Planta Med. (1986) 278–284.
[30] D.G. O'Sullivan, P.W. Sadler, C. Webley, Chemotherapy 59 (1963) 17–26.
[31] M.O. Albers, T.V. Ashworth, H.E. Oosthuizen, E. Singleton, Inorg. Synth. 26 (1989) 68.
[32] Y. Tain, C. Duan, C. Zhao, X. You, T.C.W. Mak, Z. Zhang, Inorg. Chem. 36 (1997) 1247–1252.
[33] J.R. Cedeno, D.J. Krogstad, J. Infect. Dis. 148 (6) (1983) 1090–1095.
[34] A.S. Dobek, D.L. Klayman, J.R.E.T. Dickson, J.P. Scvill, E.C. Tramont, Antimicrob. Agents Chemother. 28 (1980) 27–36.