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Synthesis, spectral studies and in vitro assessment for antiamoebic activity of new cyclooctadiene ruthenium(II) complexes with 5-nitrothiophene-2-carboxaldehyde thiosemicarbazones

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Abstract—We report here the synthesis, characterization and in vitro antiamoebic activity of 5-nitrothiophene-2-carboxaldehyde thiosemicarbazones (TSC), **1**–**5**, and their bidentate complexes $[Ru(\eta^4\text{-}C_8H_{12})(TSC)Cl_2]$ **1a**–**5a**. The biological studies of these compounds were investigated against *HK-9* strain of *Entamoeba histolytica* and the concentration causing 50% cell growth inhibition (IC_{50}) was calculated in the micromolar range. The ligands exhibited antiamoebic activity in the range (2.05–5.29 μM). Screening results indicated that the potencies of the compounds increased by the incorporation of ruthenium(II) in the thiosemicarbazones. The complexes **1a**–**5a** showed antiamoebic activity with an IC_{50} of 0.61–1.43 μM and were better inhibitors of growth of *E. histolytica*, based on IC_{50} values. The most promising among them is Ru(II) complex **2a** having 1,2,3,4-tetrahydroquinoline as N^4 substitution.

Amebiasis can be considered the most aggressive disease of the human intestine, responsible in its invasive form for clinical syndromes, ranging from the classic dysentery of acute colitis to extra-intestinal disease.[1] The protozoan parasite *Entamoeba histolytica* causes amebic colitis and amebic liver abscess, diseases that afflict millions of individuals in developing countries.[2,3] Approximately 90% of patients who present with mild-to-moderate amebic dysentery have a response to nitroimidazole therapy and metronidazole is the drug of choice for amoebiasis. However, in many cases, parasite persists in the intestine of patients and resistance to metronidazole in many pathogenic bacteria and protozoa[4,5] is known. Therefore, the need for new drugs with similar therapeutic activity but lower toxicity prevails.

Thiosemicarbazones are a class of small molecules that have been evaluated for their chemotherapeutic activity against many diseases.[6–11] The search for new antiamoebic agents has led to the examination of representative 5-nitrothiophene-2-carboxaldehyde thiosemicarbazones. These ligands coordinate with metal ions inside the cell and act as true active species. Ru(II) complexes are currently used as antileukaemic and antiviral, agents and as treatment against several types of other serious disorders such as Crohn's disease.[12–15] Complexation with the metal protects the drug against enzymatic degradation because of the inertness of certain metal–ligand linkages. Therefore, the activity can be reinforced by the combination of effects from ligands and metal residue.[16–18] We have earlier reported the synthesis and antiamoebic activity of 5-nitrothiophene thiosemicarbazones, their palladium(II) and ruthenium(II) complexes, and found very promising results,[19–21] the compounds found active, their in vivo and cytotoxicity studies are in progress. In continuation of our research to find small molecule inhibitors for the parasite *E. histolytica*, we report here the synthesis of 5-nitrothiophene-2-carboxaldehyde thiosemicarbazones and their Ru(II) complexes. In vitro antiamoebic activity of these compounds was carried out against *HK-9* strain of *E. histolytica* for their ability to inhibit the growth of parasite, which showed that the chelation induces significant changes in the antiamoebic activity.

The synthesis of 5-nitrothiophene-2-carboxaldehyde thiosemicarbazones **1**–**5** and their Ru(II) complexes **1a**–**5a** is outlined in Scheme 1. All the cycloaminothiocarbonyl

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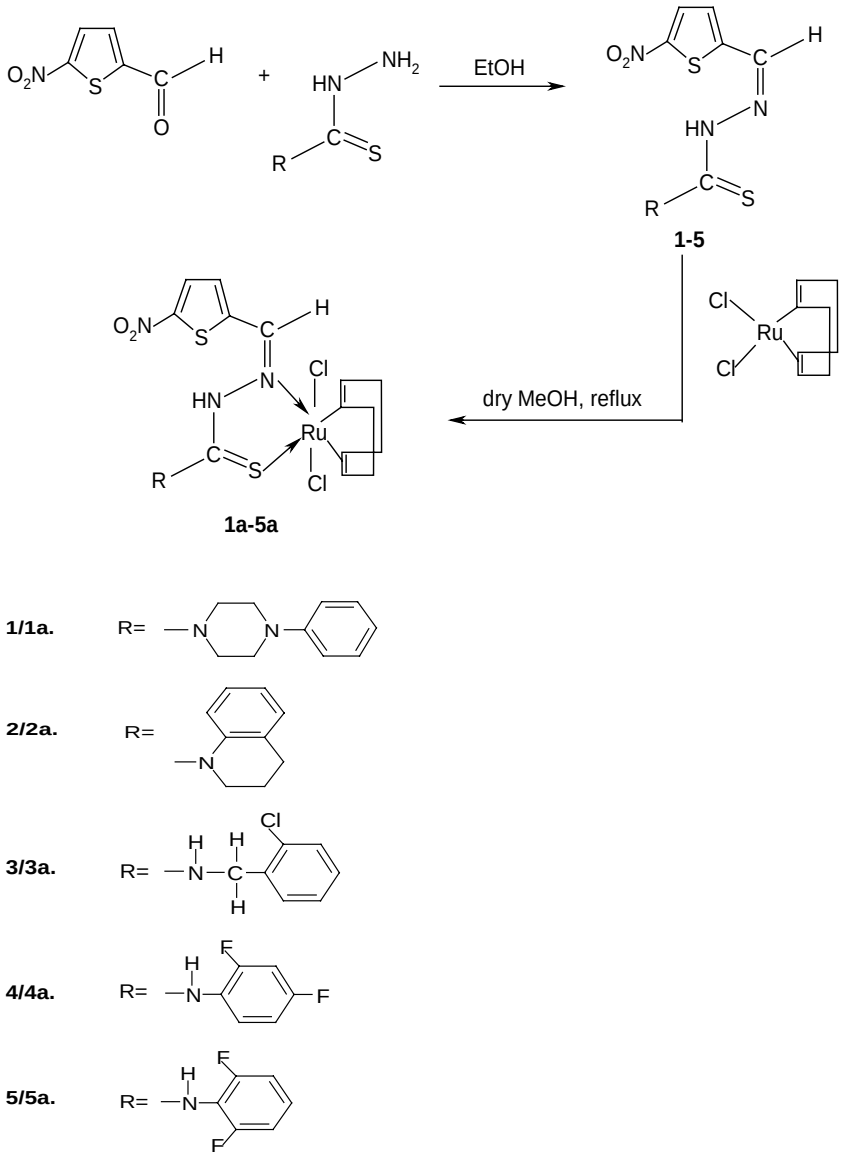

Scheme 1.

hydrazines were N^4-substituted with aromatic amines and were prepared by the literature method.[22] Condensation of cycloaminothiocarbonyl hydrazines in water with 5-nitrothiophene-2-carboxaldehyde in ethanol at 25 °C, for 3 h, afforded 5-nitrothiophene-2-carboxaldehyde thiosemicarbazones in 63–71% yield. After cooling, all the compounds were filtered and purified by crystallization from appropriate solvent. The product of the Ru(II) complexes was obtained by refluxing 1 equiv each of the free ligands and $[Ru(\eta^4\text{-}C_8H_{12})(CH_3CN)_2Cl_2]$ in dry methanolic solution for 4 h. 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. The precursor $[Ru(\eta^4\text{-}C_8H_{12})(CH_3CN)_2Cl_2]$ used for the synthesis of Ru(II) complexes was synthesized by the literature procedure.[23] The structure of all the complexes **1a**–**5a** was established by comparing the spectral data IR, UV–vis and ^1H NMR with their respective ligands **1**–**5**.[24,25] The complexes were additionally characterized by thermogravimetric analysis. The analytical data of these compounds are in agreement with their composition Table 1.

The IR spectra show band in the region 3205–3309 cm^{-1} due to stretching frequencies for NH. No band due to the SH group is observed between 2500 and 2600 cm^{-1}, in agreement with the thione form of the ligand and with the presence of band in the region 1017–1034 cm^{-1} for ν(C=S). A band for ν(C=N) appears in the region 1598–1625 cm^{-1} in all the ligands. In all the complexes, NH band shifts slightly due to probable adjustment of current arising due to coordination of thionic sulfur. The (C=S) and (C=N) band shift to lower frequencies was observed due to coordinate covalent bonding of ligands resulting in deprotonation and bonding through S and N. The preferential coordination of thionic sulfur over sulfur of thiophene is due to more nucleophilic character of the former. The band due to ν(C–S–C) (ring) of thiophene moiety remains unaltered

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

S. no.	Compound/stoichiometry	Yield (%)	Mp/dec temp (°C)	Found (calcd)			
				C	H	N	Cl
1	5-N-2-TCA-NPPTSCN $C_{16}H_{17}N_5S_2O_2$	63	165	51.34 (51.18)	4.29 (4.56)	18.71 (18.65)	—
1a	$[Ru(\eta^4\text{-}C_8H_{12})(5\text{-N-2-TCA-NPPTSC})Cl_2]$ $C_{24}H_{29}N_5S_2O_2Cl_2Ru$	64	275	44.05 (43.97)	4.29 (4.46)	10.64 (10.68)	11.06 (10.81)
2	5-N-2-TCA-1,2,3,4-THQTSCN $C_{15}H_{14}N_4S_2O_2$	65	174	51.93 (52.01)	3.85 (4.07)	16.39 (16.17)	—
2a	$[Ru(\eta^4\text{-}C_8H_{12})(5\text{-N-2-TCA-1,2,3,4-THQTSC})Cl_2]$ $C_{23}H_{26}N_4S_2O_2Cl_2Ru$	61	284	44.15 (44.09)	3.21 (4.18)	8.76 (8.94)	11.29 (11.32)
3	5-N-2-TCA-2-CBZTSCN $C_{13}H_{11}N_4S_2O_2Cl$	71	218	44.21 (44.01)	2.95 (3.12)	15.91 (15.79)	—
3a	$[Ru(\eta^4\text{-}C_8H_{12})(5\text{-N-2-TCA-2-CBTSC})Cl_2]$ $C_{21}H_{23}N_4S_2O_2Cl_3Ru$	49	280	39.59 (39.72)	3.72 (3.65)	8.91 (8.82)	16.84 (16.75)
4	5-N-2-TCA-2,4-DFATSCN $C_{12}H_8N_4S_2O_2F_2$	59	178	42.16 (42.10)	2.27 (2.36)	16.51 (16.37)	—
4a	$[Ru(\eta^4\text{-}C_8H_{12})(5\text{-N-2-TCA-2,4-DFATSC})Cl_2]$ $C_{20}H_{20}N_4S_2O_2F_2Cl_2Ru$	64	274	38.67 (38.59)	3.06 (3.24)	8.89 (9.00)	11.58 (11.39)
5	5-N-2-TCA-2,6-DFATSCN $C_{12}H_8N_4S_2O_2F_2$	67	208	41.97 (42.10)	2.19 (2.36)	16.59 (16.37)	—
5a	$[Ru(\eta^4\text{-}C_8H_{12})(5\text{-N-2-TCA-2,6-DFATSC})Cl_2]$ $C_{20}H_{20}N_4S_2O_2F_2Cl_2Ru$	61	283	38.51 (38.59)	3.14 (3.22)	9.10 (9.00)	11.23 (11.39)

in **1a**–**5a**, indicating non-participation of ring sulfur in coordination.

The electronic spectra of all the thiosemicarbazones showed a similar pattern, exhibiting three bands in the region 28752–30120, 37945–38840 and 47619–48490 cm^{-1}. The probable assignment for these bands is due to the $n \rightarrow \pi^*$ (thiosemicarbazones), $n \rightarrow \pi^*$ (thiophene) and $\pi \rightarrow \pi^*$ (thiophene) transitions. A careful comparison of the bands of electronic spectral bands of complexes with those of the free ligands showed that there was little change in the energy of these bands due to extended conjugation of ligands after complexation. In the spectra of complexes, these bands appeared at ca. 25000, 37000 and 49000 cm^{-1}, respectively. A very intense band at ca. 21500 cm^{-1} in the electronic spectra of the complexes is reasonably assignable to a combination of ligand to metal charge transfer and metal d–d band transitions.

Further evidence for the coordinating mode of the thiosemicarbazones **1**–**5** was obtained by ^1H NMR spectra. In the ^1H NMR spectra of all the thiosemicarbazones **1**–**5** in DMSO-d_6 do not show any resonance at ca. 4.0 ppm which attribute to –SH proton resonance, while the appearance of a broad peak at 9.35–11.02 ppm due to –NH proton indicates that even in a polar solvent such as DMSO they remain in the thione form. The –NH proton signal of the thiosemicarbazones usually shifts to upfield and appears at 3.49–4.32 ppm in their respective complexes. However, in some complexes, we are unable to locate the –NH proton signal. This either merges with aromatic protons or resonate beyond 15 ppm. This information suggests the adjustment of electronic current upon coordination of >C═S group to the metal ion. Other protons viz. CH_3 protons, CH_2 protons and aryl protons in complexes resonate nearly in the same region as that of free ligands.

The thermogravimetric analysis profiles of complexes **la**–**5a** under nitrogen along with the percentage weight at different temperatures are recorded. These complexes do not lose weight up to 235 °C. Further increment of temperature causes decomposition of the complexes in two steps. The temperature range for the first step being 235–410 °C for the ruthenium(II) complexes where loss of mixed fragments was observed. The second step starts immediately after first one and continues until complete decomposition of the ligand and formation of RuS as the end product. Although decomposed fragments of the ligands could not be approximated due to continuous weight loss, the total percentage of weight loss corresponds to the loss of the respective ligand after considering the transfer of one sulfur atom to the ruthenium and residue corresponds to the metal sulfide.

In this study, 5-nitrothiophene-2-carboxaldehyde thiosemicarbazones **1**–**5** and their Ru(II) complexes **1a**–**5a** were synthesized and tested as inhibitors of the parasite *E. histolytica* by microdilution method.[26] They were assessed in vitro against *Hk-9* strain of *E. histolytica* and the concentration (in micromolar) causing the 50% cell growth inhibition (IC_{50}) relative to control was calculat-

Table 2. In vitro screening of antiamoebic activities of thiosemicarbazones and their Ru(II) complexes against *Hk-9* strain of *Entamoeba histolytica*

S. no.	Compound	IC_{50} (μM)	SD*
1	5-N-2-TCA-NPPTSC	4.40	0.624
1a	$[Ru(\eta^4\text{-}C_8H_{12})$(5-N-2-TCA-NPPTSC)$Cl_2]$	1.16	0.123
2	5-N-2-TCA-1,2,3,4-THQTSC	2.05	0.196
2a	$[Ru(\eta^4\text{-}C_8H_{12})$(5-N-2-TCA-1,2,3,4-THQTSC)$Cl_2]$	0.61	0.084
3	5-N-2-TCA-2-CBTSC	4.48	0.550
3a	$[Ru(\eta^4\text{-}C_8H_{12})$(5-N-2-TCA-2-CBTSC)$Cl_2]$	1.43	0.135
4	5-N-2-TCA-2,4-DFATSC	5.29	0.839
4a	$[Ru(\eta^4\text{-}C_8H_{12})$(5-N-2-TCA-2,4-DFATSC)$Cl_2]$	1.40	0.151
5	5-N-2-TCA-2,6-DFATSC	4.59	0.692
5a	$[Ru(\eta^4\text{-}C_8H_{12})$(5-N-2-TCA-2,6-DFATSC)$Cl_2]$	1.19	0.138
	Metronidazole	1.87	0.455

* Standard deviation.

ed. The results are calculated in Table 2. Metronidazole was used as a reference amoebicidal drug and had an inhibitory concentration (IC_{50}) of 1.87 μM obtained against *E. histolytica*.[27] 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 biological test was carried out using DMSO as the solvent[28,29] in which the compounds are stable. The ligands exhibited antiamoebic activity in the micromolar range (2.05–5.29 μM). Incorporation of Ru(II) metal was found to be important for potent antiamoebic activity in the series. The complexes **1a**–**5a** showed antiamoebic activity with an IC_{50} of 0.61–1.43 μM were better inhibitors of growth of *E. histolytica*, based on IC_{50} values. The most promising among them is Ru(II) complex **2a** having 1,2,3,4-tetrahydroquinoline as N^4 substitution. The results were statistically evaluated by analysis of variance. The null hypothesis was tested using *t* test. The significance of the difference between the IC_{50} values of metronidazole and the complexes **1a**–**5a** was evaluated by *t* test. The values of the calculated *t* were found to be higher than the table value of *t* at the 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 the metal precursor has no activity against *E. histolytica*. The results indicate that the complexation to Ru increases the activity of parent ligands. All the Ru(II) complexes showed better amoebicidal activity as compared to their ligands. Detailed studies on the mechanism of action of these compounds as well as further modifications of these and other related thiosemicarbazones are in progress.

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24. 5-Nitrothiophene-2-carboxaldehyde-*N*(4)phenyl piperazine thiosemicarbazones, 5-N-2-TCA-NPPTSCN (**1**): yellow solid (acetone); UV–vis: ν (cm^{-1}) 29069, 37453, 48650; IR: ν_{max} (cm^{-1}) 3309 (NH), 1598 (C=N), 1552 (C=C), 1094 (C–N), 1017 (C=S), (N–N); ^{1}H NMR ($CDCl_3$): (δ, ppm) 10.05 (1H, s, –NH), 8.97 (1H, s, –CH=N), 4.16 (8H, m, $–CH_2$), 6.94–7.41 (7H, m, aryl).
5-Nitrothiophene-2-carboxaldehyde-*N*(4)-1,2,3,4-tetrahydroquinoline thiosemicarbazones, 5-N-2-TCA-1,2,3,4-THQTSCN (**2**): yellow solid (methanol:chloroform); UV–vis: ν (cm^{-1}) 30581, 37174, 47666; IR: ν_{max} (cm^{-1}) 3205 (NH), 1599 (C=N), 1498 (C=C), 1095 (C–N), 1027 (C=S), (N–N); ^{1}H NMR ($CDCl_3$): (δ, ppm) 10.59 (1H, s, –NH), 9.31 (1H, s, –CH=N), 3.87 (6H, m, $–CH_2$), 7.16–7.62 (6H, m, aryl).
5-Nitrothiophene-2-carboxaldehyde-*N*(4)-2-chlorobenzyl thiosemicarbazones, 5-N-2-TCA-2-CBZTSCN (**3**): yellow solid (methanol); UV–vis: ν (cm^{-1}) 28571, 36722, 47853; IR: ν_{max} (cm^{-1}) 3227 (NH), 1625 (C=N), 1544 (C=C), 1128 (C–N), 1024 (C=S), (N–N); ^{1}H NMR ($CDCl_3$): (δ, ppm) 11.04 (1H, s, –NH), 8.05 (1H, t, –NH), 9.14 (1H, s, –CH=N), 4.51 (2H, d, $–CH_2$), 7.05–7.54 (6H, m, aryl).
5-Nitrothiophene-2-carboxaldehyde-*N*(4)-2,4-difluoroaniline thiosemicarbazones, 5-N-2-TCA-2,4-DFATSCN (**4**): brick red solid (methanol); UV–vis: ν (cm^{-1}) 28985, 37965, 48683; IR: ν_{max} (cm^{-1}) 3290 (NH), 1602 (C=N), 1538 (C=C), 1140 (C–N), 1034 (C=S), (N–N); ^{1}H NMR ($CDCl_3$): (δ, ppm) 10.50 (2H, s, –NH), 8.87 (1H, s, –CH=N), 6.92–7.61 (5H, m, aryl).
5-Nitrothiophene-2-carboxaldehyde-*N*(4)-2,6-difluoroaniline thiosemicarbazones, 5-N-2-TCA-2,6-DFATSCN (**5**): light brown solid (methanol); UV–vis: ν (cm^{-1}) 28985, 37087, 48683; IR: ν_{max} (cm^{-1}) 3250 (NH), 1615 (C=N), 1542 (C=C), 1101 (C–N), 1034 (C=S), (N–N); ^{1}H NMR ($CDCl_3$): (δ, ppm) 10.79 (2H, s, –NH), 9.20 (1H, s, –CH=N), 7.04–7.53 (5H, m, aryl).
25. Dichloro (5-nitrothiophene-2-carboxaldehyde-*N*(4)phenyl piperazine thiosemicarbazones) cyclooctadiene ruthenium(II), [Ru(η^4-C_8H_{12})(5-N-2-TCA-NPPTSC)Cl_2] (**1a**): dark brown solid (methanol:DMSO); UV–vis: ν (cm^{-1}) 21975, 25470, 36454, 49217; IR: ν_{max} (cm^{-1}) 3478 (NH), 1617 (C=N), 1557 (C=C), 1012 (C=S), (N–N), 513, 489, 437 (Ru–N, Ru–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 4.02 (1H, s, –NH), 8.29 (1H, s, CH=N), 3.41 (8H, s, $–CH_2$), 2.59 (4H, m, *exo* CH_2), 2.13 (4H, m, *endo* CH_2), 4.9–5.2 (4H, m, –CH=CH–), 7.19–7.63 (7H, m, aryl).
Dichloro (5-nitrothiophene-2-carboxaldehyde-*N*(4)-1,2,3,4-tetrahydroquinoline thiosemicarbazones) cyclooctadiene ruthenium(II) [Ru(η^4-C_8H_{12})(5-N-2-TCA-1,2,3,4-THQTSC)Cl_2] (**2a**): brown solid (methanol:DMSO); UV–vis: ν (cm^{-1}) 22017, 36311, 49298; IR: ν_{max} (cm^{-1}) 3176 (NH), 1633 (C=N), 1511 (C=C), 1012 (C=S), (N–N), 517, 478, 443 (Ru–N, Ru–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 4.31 (1H, s, –NH), 8.13 (1H, s, –CH=N), 3.39 (6H, m, $–CH_2$), 2.43 (4H, m, *exo* CH_2), 2.09 (4H, m, *endo* CH_2), 5.0–5.3 (4H, m, –CH=CH–), 6.93–7.27 (6H, m, aryl).
Dichloro (5-nitrothiophene-2-carboxaldehyde-*N*(4)-2-chlorobenzyl thiosemicarbazones) cyclooctadiene ruthenium(II) [Ru(η^4-C_8H_{12})(5-N-2-TCA-2-CBTSC)Cl_2] (**3a**): dark brown solid (methanol:DMSO); UV–vis: ν (cm^{-1}) 21889, 26316, 35715, 49654; IR: ν_{max} (cm^{-1}) 3252 (NH), 1622 (C=N), 1551 (C=C), 1019 (C=S), (N–N), 485, 437 (Ru–N, Ru–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 4.40 (1H, s, –NH), 8.35 (1H, s, –CH=N), 3.48 (2H, d, J = 7.2 Hz, $–CH_2$), 2.53 (4H, m, *exo* CH_2), 2.29 (4H, m, *endo* CH_2), 4.9–5.2 (4H, m, –CH=CH–) 7.19–7.46 (6H, m, aryl).
Dichloro (5-nitrothiophene-2-carboxaldehyde-*N*(4)-2,4-difluoroaniline thiosemicarbazones) cyclooctadiene ruthenium(II)[Ru(η^4-C_8H_{12})(5-N-2-TCA-2,4-DFATSC)Cl_2] (**4a**): dark brown solid (methanol:DMSO); UV–vis: ν (cm^{-1}) 21797, 36140, 49223; IR: ν_{max}(cm^{-1}) 3346 (NH), 1627 (C=N), 1545 (C=C), 1015 (C=S), (N–N), 518, 479, 452 (Ru–N, Ru–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 4.21 (1H, s, –NH), 8.64 (1H, s, –CH=N), 2.79 (4H, m, *exo* CH_2), 2.23 (4H, m, *endo* CH_2), 4.6–5.1 (4H, m, –CH=CH–), 7.18–7.43 (5H, m, aryl).
Dichloro (5-nitrothiophene-2-carboxaldehyde-*N*(4)-2,6-difluoroaniline thiosemicarbazones) cyclooctadiene ruthenium(II), [Ru(η^4-C_8H_{12})(5-N-2-TCA-2,6-DFATSC)Cl_2] (**5a**): dark brown solid (methanol:DMSO); UV–vis: ν (cm^{-1}) 21905, 25868, 36578, 49607; IR: ν_{max} (cm^{-1}) 3276 (NH), 1631 (C=N), 1531 (C=C), 1018 (C=S), (N–N), 509, 471, 439 (Ru–N, Ru–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 4.03 (1H, s, –NH), 8.51 (1H, s, –CH=N), 2.61 (4H, m, *exo* CH_2), 2.28 (4H, m, *endo* CH_2), 5.3–5.4 (4H, m, –CH=CH–), 7.17–7.39 (5H, m, aryl).
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