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Novel bidentate complexes of Cu(II) derived from 5-nitrofuran-2-carboxaldehyde thiosemicarbazones with antiamoebic activity against *E. histolytica*

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Abstract

The novel analogues of 5-nitrofuran-2-carboxaldehyde thiosemicarbazones **1**–**10** were synthesized and their copper(II) complexes **1a**–**10a** were obtained by means of coordination with cupric chloride. All these compounds have been characterized by elemental analysis, IR, electronic spectra and thermogravimetric patterns while ligands have also been characterized by ^{1}H NMR spectral studies. These copper complexes are bidentate and possess octahedral geometry around Cu(II) ion. Their antiamoebic activities were carried out to ascertain their effectiveness in comparison to their corresponding thiosemicarbazones. A number of these complexes possess noteworthy potencies towards *HK-9* strain of *Entamoeba histolytica* in vitro. The complexes **2a**–**7a**, **9a** and **10a** showed less IC_{50} value than metronidazole, the drug of choice for amoebiasis. Moreover, complexes **2a** and **9a** have shown the most promising antiamoebic activities (IC_{50} = **0.38** μM of **2a** and IC_{50} = **0.34** μM of **9a** versus IC_{50} = **1.81** μM of metronidazole). These results indicate that the metallated thiosemicarbazone may be lead molecule to inhibit growth of *E. histolytica*.

Keywords: 5-Nitrofuran-2-carboxaldehyde; Thiosemicarbazones; Copper(II) complexes; Antiamoebic activity

1. Introduction

Amoebiasis is mainly characterized by dysentery, and can progress to hepatic amoebiasis and other complications in untreated patients. Globally as many as 50 million people may harbor the parasite and 100,000 die each year as a consequence of fulminating colitis or liver abscess [1]. Metronidazole is an effective antiamoebic medication; it is mutagenic and has been associated with serious side effects [2]. Some *Entamoeba histolytica* strains resistant to this drug have also begun to appear [3].

Thiosemicarbazones and their metal complexes have been extensively studied during recent years owing to their wide variety of biological activities [4–8]. Certain drugs show even enhanced activity when administered as their metal chelates [9]. Copper(II) is a biologically active essential metal ion; its chelating ability and positive redox potential allow participation in biological transport reactions [10]. Further, copper(II) complexes possess a wide spectrum of biological activities [11]. The derivatives of 5-nitrofuran-2-carboxaldehyde possess interesting antibacterial and antifungal activity [12,13] led us to study the thiosemicarbazones and their copper(II) complexes. Earlier we have reported different heterocyclic thiosemicarbazones, their transition metal complexes and in vitro screening against *E. histolytica* [14–17]. The compounds found with less IC_{50} value than metronidazole, their in vivo and cytotoxicity studies are in progress. We report here the synthesis of 5-nitrofuran 2-carboxaldehyde thiosemicarbazones (**1**–**10**) Fig. 1 and their subsequent bidentate Cu(II) complexes (**1a**–**10a**) Fig. 2. These compounds were screened for their antiamoebic activities against *HK-9* strain of *E. histolytica* in vitro experiments and found that the coordination of copper to thiosemicarbazone enhances activity.

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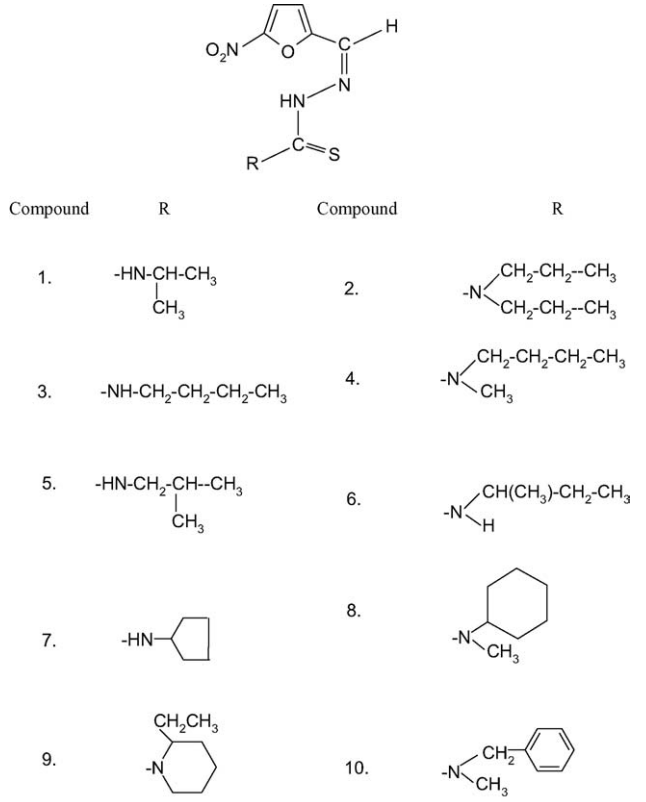

Fig. 1. Structure of thiosemicarbazones of 5-nitrofuran-2-carboxaldehyde.

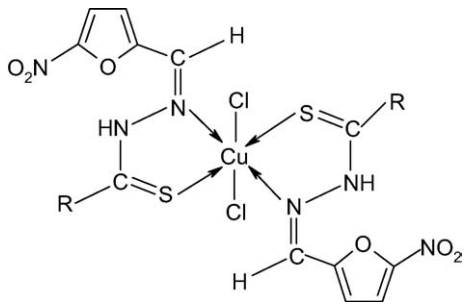

Fig. 2. Structure of copper(II) complexes.

2. Results and discussion

All the dithiocarbamic acids used for the preparation of thiocarbonylhydrazines were prepared by the method described in Ref. [18]. Thiocarbonylhydrazines were prepared by refluxing the alkaline solution of the respective dithiocarbamic acids with hydrazine hydrate. Their thiosemicarbazones were synthesized by stirring the aqueous solution of thiocarbonylhydrazines and the ethanolic solution of 5-nitrofuran-2-carboxaldehyde at ambient temperature. After cooling the reaction mixture, the solid separated was filtered and the crude mass was recrystallized from appropriate solvent. Melting point determination was carried out to check the purity of the compounds. IR, ^{1}H NMR and electronic spectral data established the structures of all ligands. All copper(II) complexes were prepared by mixing the ligand and cupric chloride in refluxing methanol. The solution was kept at 0 °C overnight where complexes were separated out. This was filtered, washed with hot water followed by minimal of methanol and dried. Recrystallization of all the complexes was carried out from methanol. All the copper(II) complexes are non-hygroscopic, stable and isolated in good yield. They are non-electrolyte in nature DMF (Λ_M = 1.5–8.0 Ω^{-1} cm^2 mol^{-1}). On heating all the complexes decomposed and did not present a clear melting point. The structure of the complexes **1a–10a** was established by comparing spectral data with the free ligand along with their thermogravimetric patterns.

2.1. ^{1}H NMR spectral studies of ligands

^{1}H NMR spectra of all the ligands have been taken in DMSO/$CDCl_3$ and that also favors the proposed structures. In all the ligands (NH) protons appears at (8.30–10.30) ppm. Other NH proton could not be located in the 0–15 ppm due to possible exchange of NH proton with deuterated solvent. Two doublets in the range (6.80–7.24) ppm and (7.38–7.63) were assigned to the furan protons in all the ligands. A singlet also appears at (7.55–8.55) which was assigned to the (CH=N) protons.

2.2. IR and electronic spectral studies of ligands and their complexes

The interest in the IR spectra of the bidentate nitrogen–sulfur donor ligands lie mainly in the bands due to (NH–C=S) group and (C=N) group. All the ligands may exist in thione-thiol tautomerization since they contain a thioamide (–HN–C=S) functional group. However, due to absence of the ν(S–H) stretch in the region 2500–2600 cm^{-1} and presence of ν(N–H) stretch in the region 3137–3312 cm^{-1} in the IR spectra of ligands indicate that all the ligands retain their thione form in the solid state. This is further inferred from the presence of a strong band in the region 1031–1099 cm^{-1} due to the ν(C=S) stretch.

A strong band appearing in the region 1600–1647 cm^{-1} is assigned to the ν(C=N) stretch and shift of this band by 15–41 cm^{-1} to lower frequency indicates the involvement of azomethine nitrogen in complexation. The band due to (HN–C=S) group is shifted to lower frequency there by indicating the involvement of the thione sulfur in complex formation. This contention is further confirmed by the presence of ν(Cu–N) and ν(Cu–S) bands at (510–565) and (438–448) cm^{-1} in the far IR frequency region of the complexes.

The electronic spectra of these complexes exhibited bands at expected position as sighted in the literature for the similar system [19]. A perusal of these spectra revealed that they were dominated by intense intraligand and charge transfer bands. Thus a band appearing at (244–250) nm in the spectra of complexes was assigned to π–π* transition of furan ring. Another band at (317–328) nm was attributed to n–π* transitions of furan ring. This band was followed by a shoulder band at (398–402) nm appearing due to the thiosemicarbazones moi-

ety. A comparison of these three bands with free ligands revealed that there is increase in intensity and decrease in the frequency which is attributed to extended conjugation in the ligand moieties after complexation. The complexes show absorption peaks in the visible region due to the d–d transition of the single d electron of the copper(II) ion. All the complexes exhibited two bands in the regions (313–361) and (617–877) nm attributed to six coordinate geometry of copper(II) complexes.

On the basis of IR and electronic spectra an octahedral geometry for all the complexes has been proposed.

2.3. Thermogravimetric studies

The structural characteristics of these complexes were further delivered by a careful examination of their thermogravimetric patterns. The thermal decomposition pattern of these complexes showed that they have stability range from ambient temperature to 150 °C. The thermograms of some of the complexes represented a two stage decomposition while others represent a three stage decomposition. In the two stage decomposition the first stage generally occurred in the temperature range of 240–300 °C presumably due to loss of chloride and mixed ligand fragments as total loss in this fragment always weigh much higher than expected for two chloride. The second stage that started immediately after the first one occurred in the temperature range of 340–410 °C and continued until the complete decomposition of the ligands ultimately leaving copper oxide as the residue. In the three-stages decomposition the third stage occurred in the range 610–720 °C exhibiting complete decomposition of the ligand associated with the complex.

2.4. Biological activity

All the compounds were evaluated for antiamoebic activity in vitro using *HK-9* strain of *E. histolytica*. The IC_{50} values in μM are shown in Table 1. 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 free ligands **1–10** exhibited antiamoebic activity with IC_{50} of 2.39–11.45 μM. Considering the substitutions at N^4 position in thiosemicarbazones with *N*-methylbutylamine (**4**) and 2-ethylpiperidine (**9**) showed less (IC_{50} = 2.39, 2.68 μM, respectively) as compared to other ligands. Complexation of the thiosemicarbazones with copper(II) results in compounds (**1a**–**10a**), which showed improvement in antiamoebic activity (IC_{50} = 0.34–2.85 μM) as compared to their respective ligands and the copper complexes **2a**–**7a**, **9a** and **10a** showed better IC_{50} value than metronidazole in vitro. 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 MNZ and the complexes **2a**–**7a**, **9a** and **10a** was evaluated by *t*-test.

Table 1
In vitro antiamoebic activities of 5-nitrofuran-2-carboxaldehyde thiosemicarbazones and their Cu(II) complexes against *HK-9* strain of *E. histolytica*

Compound	IC_{50} (μM)	S.D.[a]
1	11.45	2.23
1a	2.85	4.41
2	6.11	1.37
2a	0.38	0.60
3	6.59	1.29
3a	1.56	0.34
4	2.39	0.63
4a	1.02	0.24
5	8.11	1.93
5a	1.39	0.38
6	8.70	1.74
6a	1.54	0.41
7	5.00	1.17
7a	0.93	0.21
8	9.84	1.52
8a	2.60	0.59
9	2.68	0.45
9a	0.34	0.15
10	4.90	0.91
10a	1.15	0.26
$CuCl_2{\cdot}2H_2O$	5.60	0.15
Metronidazole	1.81	0.47

[a] S.D., standard deviation.

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 complexation enhances the activity of the ligand, it may be due to chelation, which reduces the polarity of the central metal atom because of partial sharing of its positive charge with the ligand, which favors permeation of the complexes through the lipid layer of cell membrane [20]. The Cu-complex precursor $CuCl_2{\cdot}2H_2O$ was also evaluated for antiamoebic activity and compared with Cu(II) complexes and metronidazole, which showed no activity against *E. histolytica*. It is concluded that the presence of these bulky groups at position N^4 of the thiosemicarbazone moiety enhanced antiamoebic activity. 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 [21].

3. Conclusion

This research examined the biological activities of the new thiosemicarbazones prepared from 5-nitrofuran-2-carboxaldehyde and their copper(II) complexes. *HK-9* strain of *E. histolytica* was employed for in vitro antiamoebic evaluation. The biological behavior revealed that most of the ligands show a weak activity against *E. histolytica*. The chelation induced significant changes in the biological activity of the ligands and the copper complexes **2a**–**7a**, **9a** and **10a** have shown better IC_{50} value than metronidazole in vitro. The complexes

2a and **9a** have shown the most promising antiamoebic activities. Detailed studies on the mechanism of action of these complexes as well as further modifications of these and other related metal derivatives are in progress.

4. Experimental protocols

4.1. Chemistry

Copper chloride was purchased from S.D. fine chemicals company (India). All the thiocarbonylhydrazines were prepared as reported earlier [18]. Elemental analysis (C, H, N) was carried out by Central Drug Research Institute, Lucknow, India. Chlorine was estimated by standard method. Melting points were recorded on a KSW melting point apparatus and were uncorrected. Electronic spectra were recorded in DMF on a Shimadzu UV-1601 PC UV–Visible spectrophotometer. Magnetic moment measurement of the copper(II) complexes were carried out by the institute instrumentation center of Indian Institute of Technology, Roorkee, India. Electric conductance measurements of the complexes were done in 10^{-3} M solutions of DMF using Biochem model DC 808 digital conductivity bridge calibrated with potassium chloride solution. IR spectra were recorded as KBr disks on a Perkin Elmer model 1620 FT-IR spectrophotometer. ^{1}H NMR spectra were obtained at ambient temperature using a Bruker spectrospin DPX-300 MHz instrument in $CDCl_3$ and DMSO-d_6 using tetramethylsilane as an internal standard. Thermograms of the complexes were recorded under nitrogen on a TG 51 thermogravimetric analyzer with increasing the temperature at 10 °C min^{-1}.

4.1.1. Synthesis of 5-nitrofuran-2-carboxaldehyde thiosemicarbazones

All thiosemicarbazones were synthesized by mixing an aqueous solution of thiocarbonylhydrazines (0.003 mol in 10 ml) and ethanolic solution of 5-nitrofuran-2-carboxaldehyde (0.003 mol in 10 ml) at 25 °C for 3 h with continuous stirring. After cooling at ca. 10 °C for 24 h, the precipitated compound was filtered and recrystallized from appropriate solvent.

*4.1.1.1. 5-Nitrofuran-2-carboxaldehyde-N_4-isopropylthiosemicarbazone (**1**).* Orange crystals (chloroform). Yield: 60%; m.p.: 187 °C. Anal. Calc. for $C_9H_{12}N_4O_3S$: C 42.19, H 4.69, N 21.87 %; found: C 42.54, H 4.70, N 21.42 %; UV–Vis: λ_{max}/nm: 225, 283, 331; IR: ν_{max}/cm^{-1}: 3165 (NH), 1620 (C=N), 1099 (C=S); ^{1}H NMR ($CDCl_3$): δ/ppm: 1.35 (6H, d, –CH_3, J = 5.4), 4.59–4.61 (1H, m, –CH, J = 7.2), 6.88 (1H, d, furan, J = 3.5), 7.38 (1H, d, furan, J = 3.5), 7.62 (1H, s, –CH=N), 9.4 (1H, s, –NH).

*4.1.1.2. 5-Nitrofuran-2-carboxaldehyde-N_4-bis(propyl)thiosemicarbazone (**2**).* Dark yellow crystals (methanol). Yield: 85%; m.p.: 139 °C. Anal. Calc. for $C_{12}H_{18}N_4O_3S$: C 48.32, H 6.04, N 18.79%; found: C 48.42, H 6.06, N 18.24%; UV–Vis: λ_{max}/nm: 227, 286, 335; IR: ν_{max}/cm^{-1}: 3168 (NH), 1605 (C=N), 1100 (C=S); ^{1}H NMR($CDCl_3$): δ/ppm: 1.58 (6H, t, –CH_3, J = 6.6), 2.47–2.66 (8H, m, CH_2, J = 12.5), 7.16 (1H, d, furan, J = 3.6), 7.39 (1H, d, furan, J = 3.5), 8.29 (1H, s, –CH=N), 10.4 (1H, s, –NH).

*4.1.1.3. 5-Nitrofuran-2-carboxaldehyde-N_4-butylthiosemicarbazone (**3**).* Yellow crystals (methanol). Yield: 85%; m.p.: 137 °C. Anal. Calc. for $C_{10}H_{14}N_4O_3S$: C 44.81, H 5.22, N 20.6%; found: C 44.60, H 5.01, N 20.84%; UV–Vis: λ_{max}/nm: 226, 288, 335; IR: ν_{max}/cm^{-1}: 3148 (NH), 1638 (C=N), 1117 (C=S); ^{1}H NMR ($CDCl_3$): δ/ppm: 1.07 (3H, t, –CH_3, J = 6.5), 1.53 (2H, m, –CH_2, J = 4.6), 1.79 (2H, m, –CH_2, J = 8.9), 3.80 (2H, t, –CH_2, J = 7.9), 6.90 (1H, d, furan, J = 3.6), 7.46 (1H, d, furan, J = 3.6), 7.90 (1H, s, –CH=N), 10.5 (1H, s, –NH).

*4.1.1.4. 5-Nitrofuran-2-carboxaldehyde-N_4-methylbutylthiosemicarbazone (**4**).* Yellow crystals (methanol), Yield: 62%; m.p.: 159 °C. Anal. Calc. for $C_{11}H_{16}N_4O_3S$: C 46.48, H 5.63, N 19.72%; found: C 46.98, H 5.95, N 19.89%; UV–Vis: λ_{max}/nm: 227, 285, 332; IR: ν_{max}/cm^{-1}: 3136 (NH), 1620 (C=N), 1098(C=S), ^{1}H NMR($CDCl_3$): δ/ppm: 0.98 (3H, t, –CH_3, J = 7.1), 1.36–1.46 (2H, m, –CH_2, J = 6.3), 1.76–1.78 (2H, m, –CH_2), 3.39 (3H, s, –CH_3), 3.78 (2H, t, –CH_2, J = 9.4), 6.80 (1H, d, furan, J = 3.6), 7.37 (1H, d, furan, J = 3.6), 7.54 (1H, s, –CH=N), 8.96 (1H, s, –NH).

*4.1.1.5. 5-Nitrofuran-2-carboxaldehyde-N_4-isobutylthiosemicarbazone (**5**).* Yellow crystals (methanol), Yield: 65%; m.p.: 161 °C. Anal. Calc. For $C_{10}H_{14}N_4O_3S$: C 44.81, H 5.22, N 20.63%; found: C 44.44, H 5.19, N 20.88%; UV–Vis: λ_{max}/nm: 226, 286, 335; IR: ν_{max}/cm^{-1}: 3137 (NH), 1638 (C=N), 1107 (C=S), ^{1}H NMR($CDCl_3$): δ/ppm: (6H, d, –CH_3, J = 6.1), 2.01–2.10 (1H, m, –CH, J = 7.3), 3.57 (2H, m, –CH_2, J = 6.1), 7.20 (1H, d, furan, J = 3.5), 7.43 (1H, d, furan, J = 3.6), 8.55 (1H, s, –CH=N), 10.30 (1H, s, –NH).

*4.1.1.6. 5-Nitrofuran-2-carboxaldehyde-N_4-sec.butylthiosemicarbazone (**6**).* Yellow crystals (methanol), Yield: 65%; m.p.: 151 °C. Anal. Calc. for $C_{10}H_{14}N_4O_3S$: C 44.44, H 5.19, N 20.74%; found: C 44.17, H 5.34, N 20.87%; UV–Vis: λ_{max}/nm: 228, 287, 330; IR: ν_{max}/cm^{-1}: 3142 (NH), 1640(C=N), 1115(C=S); ^{1}H NMR($CDCl_3$): δ/ppm: 0.99 (3H, t, –CH_3, J = 6.0), 1.44 (3H, d, –CH_3, J = 5.4), 1.65–1.75 (2H, m, –CH_2), 3.69–3.76 (1H, m, –CH, J = 7.8), 6.86 (1H, d, furan, J = 3.5), 7.38 (1H, d, furan, J = 3.5), 7.72 (1H, s, –CH=N), 9.98 (1H, s, –NH).

*4.1.1.7. 5-Nitrofuran-2-carboxaldehyde-N_4-cyclopentylthiosemicarbazone (**7**).* Light yellow crystals (methanol), Yield: 65%; m.p.: 145 °C. Anal. Calc. for $C_{11}H_{14}N_4O_3S$: C 46.81, H 4.96, N 19.86%; found: C 46.91, H 4.96, N 19.36%; UV–Vis: λ_{max}/nm: 224, 286, 335; IR: ν_{max}/cm^{-1}: 3123 (NH), 1639 (C=N), 1085 (C=S); ^{1}H NMR($CDCl_3$): δ/ppm: 1.58–

2.308H, m, –CH_2), 4.24–4.60 (1H, m, –CH, J = 3.04), 7.14 (1H, d, furan, J = 3.7), 7.40 (1H, d, furan, J = 3.7), 8.55 (1H, s, –CH=N), 10.25 (1H, s, –NH).

*4.1.1.8. 5-Nitrofuran-2-carboxaldehyde-N_4-methylcyclohexylthiosemicarbazone (**8**).* Yellow crystals (chloroform). Yield: 70%; m.p: 125 °C. Anal. Calc. for $C_{13}H_{18}N_4O_3S$: C 50.32, H 5.81, N 18.06%; found: C 50.61, H 5.88, N 18.27%; UV–Vis: λ_{max}/nm: 229, 287, 335; IR: ν_{max}/cm^{-1}: 3125 (NH), 1647 (C=N), 1140 (C=S); ^{1}H NMR($CDCl_3$): δ/ppm: 1.48–2.90 (11H, m, –N–C_6H_{11}, J = 11.7), 3.18 (3H, s, –CH_3), 7.12 (1H, d, furan, J = 3.6), 7.42 (1H, d, furan, J = 3.6), 8.05 (1H, s, –CH=N), 10.1 (1H, s, –NH).

*4.1.1.9. 5-Nitrofuran-2-carboxaldehyde-N_4-ethylpiperidinethiosemicarbazone (**9**).* Yellow crystals (methanol), Yield: 85%; m.p.: 121 °C. Anal. Calc. for $C_{13}H_{18}N_4O_3S$: C 50.32, H 5.81, N 18.07%; found: C 50.26, H 5.93, N 18.34%; UV–Vis: λ_{max}/nm: 227, 287, 335; IR: ν_{max}/cm^{-1}: 3150 (NH), 1626 (C=N), 1148(C=S); ^{1}H NMR ($CDCl_3$): δ/ppm:1.04 (3H, t, –CH_3, J = 11.5), 1.47–1.50 (2H, m, –CH_2), 1.96–1.99 (6H, m, –CH_2), 3.33–3.43 (2H, m, –CH_2), 4.06–4.10 (1H, m, –CH), 7.20 (1H, d, furan, J = 3.7), 7.49 (1H, d, furan, J = 3.7), 8.21 (1H, s, –CH=N), 10.12 (1H, s, –NH).

*4.1.1.10.5-Nitrofuran-2-carboxaldehyde-N_4-methylbenzylthiosemicarbazone (**10**).* Golden yellow crystals (chloroform). Yield: 65%; m.p.: 147 °C. Anal. Calc. for $C_{14}H_{14}N_4O_3S$: C 52.83, H 4.40, N 17.60%; found: C 52.64, H 4.37, N 17.76%; UV–Vis: λ_{max}/nm: 226, 283, 333; IR: ν_{max}/cm^{-1}: 3139 (NH), 1621(C=N), 1130 (C=S); ^{1}H NMR($CDCl_3$): δ/ppm: 3.38 (3H, s, –CH_3), 5.06 (2H, s, –CH_2), 7.24–7.42 (7H, m, aryl), 7.55 (1H, s, –CH=N), 9.14 (1H, s, –NH).

4.1.2. Cu(II) complexes of 5-nitrofuran-2-carboxaldehyde thiosemicarbazones

General procedure: To a hot solution of the appropriate ligand (4 mmol) in methanol (20 ml) was added a solution of cupric chloride (2 mmol) dissolved in minimum quantity of methanol and the reaction mixture was heated under reflux for 1–3 h. After keeping the solution at 0 °C overnight, the colored solid separated out. This was filtered off and washed with hot water followed by small quantity of methanol and dried. Recrystallization of all the complexes was carried out from methanol.

*4.1.2.1. [5-Nitrofuran-2-carboxaldehyde-N_4-isopropylthiosemicarbazone] copper(II) chloride (**1a**).* Reddish brown crystals (methanol), Yield: 65%; m.p.: 245 °C. Anal. Calc. for $C_{18}H_{24}N_8O_6S_2Cl_2Cu$: C 33.41, H 3.71, N 17.32, Cl 10.98%; found: C 33.88, H 3.80, N 17.84, Cl 11.12%; μ_{eff}/B.M.: 1.78; UV–Vis: λ_{max}/nm: 249, 320, 362, 399, 707; IR: ν_{max}/cm^{-1}: 3169 (NH), 1600 (C=N), 1040 (C=S), 437 (Cu–N), 315 (Cu–S).

*4.1.2.2. [5-Nitrofuran-2-carboxaldehyde-N_4-bis(propyl)thiosemicarbazones] copper(II) chloride (**2a**).* Dark brown crystals (methanol), Yield: 67%, m.p.: 175 °C, Anal. Calc. for $C_{24}H_{36}N_8O_6S_2Cl_2Cu$: C 39.43, H 4.93, N 15.33, Cl 9.72%; found: C 39.94, H 5.02, N 14.98, Cl 9.43%; μ_{eff}/B.M.: 1.77; UV–Vis: λ_{max}/nm: 251, 321, 327, 400, 856; IR: ν_{max}/cm^{-1} 3172 (NH), 1590 (C=N), 1048 (C=S), 439 (Cu–N), 312 (Cu–S).

*4.1.2.3. [5-Nitrofuran-2-carboxaldehyde-N_4-butylthiosemicarbazone] copper(II) chloride (**3a**).* Chocolate brown crystals (methanol), Yield: 65%; m.p.: 205 C. Anal. Calc. for $C_{20}H_{28}N_8O_6S_2Cl_2Cu$: C 35.58, H 4.15, N 16.61, Cl 10.52%, found: C 35.33, H 4.20, N 16.62, Cl 10.68%, μ_{eff}/B.M.: 1.75 UV–Vis: λ_{max}/nm: 245, 314, 319, 402, 877; IR: ν_{max}/cm^{-1}: 3144 (NH), 1610 (C=N), 1098 (C=S), 430 (Cu–N), 314 (Cu–S).

*4.1.2.4. [5-Nitrofuran-2-carboxaldehyde-N_4-methylbutylthiosemicarbazone] copper(II) chloride (**4a**).* Dark brown crystals (methanol), Yield: 70%; m.p.: 195 °C. Anal. Calc. for $C_{22}H_{32}N_8O_6S_2Cl_2Cu$: C 37.58, H 4.55, N 15.94, Cl 10.10%; found: C 37.96, H 4.25, N 16.04, Cl 10.36%; μ_{eff}/B.M.: 1.80; UV–Vis: λ_{max}/nm: 250, 318, 335, 398, 813; IR: ν_{max}/cm^{-1}: 3137 (NH), 1598 (C=N), 1098 (C=S), 438 (Cu–N), 319 (Cu–S).

*4.1.2.5. [5-Nitrofuran-2-carboxaldehyde-N_4-isobutylthiosemicarbazone] copper(II) chloride (**5a**).* Dark brown crystals (methanol), Yield: 68%; m.p.: 182 °C. Anal. Calc. for $C_{20}H_{28}N_8O_6S_2Cl_2Cu$: C 35.58, H 4.15, N 16.61, Cl 10.52%; found: C 35.88, H 4.25, N 16.24, Cl 10.47%; μ_{eff}/B.M.: 1.79; UV–Vis: λ_{max}/nm: 245, 319, 321, 398, 747; IR: ν_{max}/cm^{-1}: 3132 (NH), 1600 (C=N), 1070 (C=S), 438 (Cu–N), 316 (Cu–S).

*4.1.2.6. [5-Nitrofuran-2-carboxaldehyde-N_4-sec.butylthiosemicarbazone] copper(II) chloride (**6a**).* Brown crystals (methanol), Yield: 69%; m.p.: 196 °C. Anal. Calc. for $C_{20}H_{28}N_8O_6S_2Cl_2Cu$: C 35.58, H 4.15, N 16.61, Cl 10.52%; found: C 35.90, H 4.58, N 16.49, Cl 10.36%; μ_{eff}/B.M.: 1.77; UV–Vis: λ_{max}/nm: 249, 320, 323, 400; IR: ν_{max}/cm^{-1}: 3117 (NH), 1600 (C=N), 1098 (C=S), 442 (Cu–N), 314 (Cu–S).

*4.1.2.7. [5-Nitrofuran-2-carboxaldehyde-N_4-cyclopentylthiosemicarbazone] copper(II) chloride (**7a**).* Light brown crystals (methanol), Yield: 71%; m.p.: 215 °C. Anal. Calc. for $C_{22}H_{28}N_8O_6S_2Cl_2Cu$: C 37.80, H 4.01, N 16.03, Cl 10.16%; found: C 37.12, H 4.04, N 16.08, Cl 10.42%; μ_{eff}/B.M.: 1.78; UV–Vis: λ_{max}/nm: 244, 324, 377, 400, 719; IR: ν_{max}/cm^{-1}: 3140 (NH), 1597 (C=N), 1060 (C=S), 442 (Cu–N), 318(Cu–S).

*4.1.2.8. [5-Nitrofuran-2-carboxaldehyde-N_4-methylcyclohexylthiosemicarbazone] copper(II) chloride (**8a**).* Chocolate brown crystals (methanol), Yield: 65%; m.p.: 202 °C. Anal. Calc. for $C_{26}H_{36}N_8O_6S_2Cl_2Cu$: C 41.35, H 4.77, N 14.85, Cl 9.41%; found: C 41.95, H 4.80, N 14.92, Cl 9.87%; μ_{eff}/B.M.: 1.81; UV–Vis: λ_{max}/nm: 248, 314, 319, 328, 400; IR: ν_{max}/cm^{-1}: 3128 (NH), 1590 (C=N), 1355 (C=S), 438 (Cu–N), 310 (Cu–S).

*4.1.2.9. [5-Nitrofuran-2-carboxaldehyde-N_4-ethylpiperidine thiosemicarbazones] copper(II) chloride (**9a**).* Brown crystals (methanol), Yield: 73%; m.p.: 198 °C. Anal. Calc. for $C_{26}H_{36}N_8O_6S_2Cl_2Cu$: C 41.35, H 4.77, N 14.84, Cl 9.41%; found: C 41.45, H 4.86, N 14.63, Cl 9.18%; μ_{eff}/B.M.: 1.76; UV–Vis: λ_{max}/nm: 250, 328, 335, 401, 700; IR: ν_{max}/cm^{-1}: 3151 (NH), 1600 (C=N), 1089 (C=S), 434 (Cu–N), 310 (Cu–S).

*4.1.2.10. [5-Nitrofuran-2-carboxaldehyde-N_4-methylbenzylthiosemicarbazone] copper(II) chloride (**10a**).* Dark brown crystals (methanol), Yield: 69%; m.p.: 216 °C. Anal. Calc. for $C_{28}H_{28}N_8O_6S_2Cl_2Cu$: C 43.61, H 3.63, N 14.54, Cl 9.21%; found: C 43.52, H 3.70, N 14.17, Cl 9.45%; μ_{eff}/B.M.: 1.80; UV–Vis: λ_{max}/nm: 250, 317, 329, 400, 672; IR: ν_{max}/cm^{-1}: 3137 (NH), 1590 (C=N), 1088 (C=S), 445 (Cu–N), 318 (Cu–S).

4.2. In vitro testing against E. histolytica

The thiosemicarbazones and their Cu(II) complexes were screened in vitro for antiamoebic activity against (*HK-9*) strain of *E. histolytica* by microdilution method [22]. *E. histolytica* trophozoites were cultured in TYIS-33 growth medium as described previously [23] in wells of 96 well microtiter plate. All the compounds were dissolved in DMSO (40 µl) at which level no inhibition of amoeba occurs [24,25] and the stock solutions of the compounds were prepared freshly before use at a concentration of 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, control wells (culture medium plus amoebae) and a blank (culture medium only). The number of amoeba per ml was estimated with a haemocytometer and trypan blue exclusion was used to confirm viability. The cell suspension used 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. 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. The culture medium was removed by inverting the plate and shaking gently. Plate was then immediately washed once in 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. The plate was allowed to dry at room temperature, and the amoebae were fixed with methanol and when dry, stained with (0.5%) aqueous eosin for 15 min. Stained plate was washed once with tape water and 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. The results are reported in Table 1.

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