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Synthesis and In Vitro Antiprotozoal Activity of 5-Nitrothiophene-2-carboxaldehyde Thiosemicarbazone Derivatives

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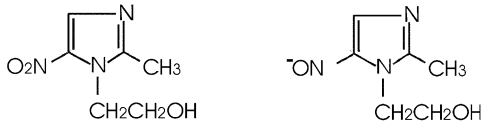
Abstract—Several thiosemicarbazone derivatives of 5-nitrothiophene-2-carboxaldehyde were prepared by the simple process in which N^4-thiosemicarbazone moiety was replaced by aliphatic, arylic and cyclic amine. Among these thiosemicarbazones compound **11** showed significant antiamoebic activity whereas compound **3** was more active antitrichomonal than the reference drug.

Amoebiasis caused by the protozoan parasite *Entamoeba histolytica* causes up to 100,000 deaths per annum[1] placing it third amongst deadly parasitic diseases. *Giardia lamblia* also called *Giardia intestinalis* is a protozoan parasite of the small intestine that causes extensive morbidity worldwide and infects approximately 2% of the adults and 6–8% of the children in developed countries.[2,3] *Trichomonas vaginalis* is a parasite protozoan that causes trichomoniasis. The main disorder produced by *T. vaginalis* is vaginalis. Metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole] (Fig. 1a) is the drug most widely used in the treatment of anaerobic protozoan parasitic infections caused by *E. histolytica*, *T. vaginalis* and *G. lamblia*.[4] In the later cases clinical resistance to metronidazole have been reported.[5,6] The mechanism of action of metronidazole affects electron transport and the chemically reactive reduced form of metronidazole (Fig. 1b) is cytotoxic to parasites.[7] It has common side effects[8] including nausea, it is mutagenic in bacteria[9] and high doses may cause cancer in rodents.[10] In addition the possibility of the future development of resistant strains, as well demonstrated by other protozoa, cannot be excluded.

A series of thiosemicarbazones derived from 2-formylpyridine, isoquinoline-1-carboxaldehyde and 2-acetylpyridine were synthesized and evaluated for antimalarial, antitrypanosomal[11,12] and antineoplastic activity.[13] Semicarbazone derivatives of 5-nitro thiophene-2-carboxaldehyde by using butyl, hexyl, morpholine, 1-methylpiperazine, 2-methoxyethyl, 2-phenylethyl and 2-(3,4-dimethoxyphenyl) amine were reported as anti-trypanosomal agents.[14] The significant antiparasitic activity of several derivatives of 5-nitrothiophene-2-carboxaldehyde[15–17] and antiprotozoan activity of 5-nitrothiophene oxime ether derivatives[18] led us to study the antiprotozoal activity of new thiosemicarbazone derivatives in the thiophene series. The study described the synthesis, structure elucidation and examined the activities of new thiosemicarbazone compounds against *E. histolytica, T. vaginalis* and G. *lamblia* the three most medically important anaerobic protozoans.



1a Metronidazole 1b Nitroso free radical

Figure 1.

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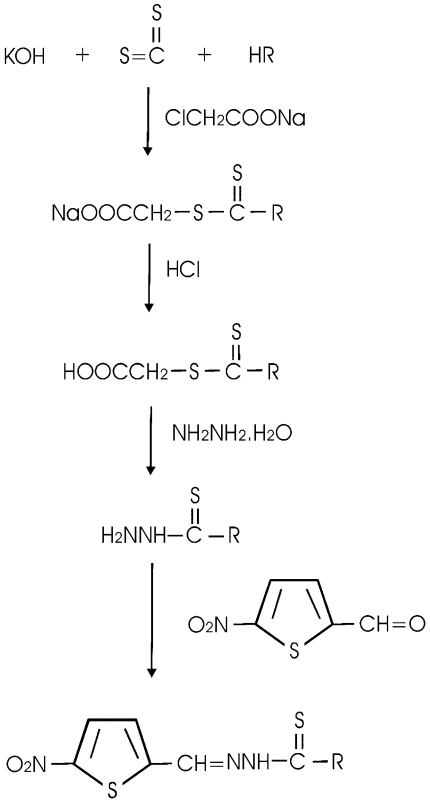
Table 1. Analytical and physicochemical data of thiosemicarbazones

S. No.	Compd/stoichiometry[a]	Colour	Yield (%)	Mp (°C)	Found (calcd) (%)		
					C	H	N
1	***5-NTPrTSC*** $C_9H_{12}N_4S_2O_2$	Reddish yellow	54	185	39.54 (39.71)	4.22 (4.41)	20.49 (20.59)
2	***5-NT-isoPrTSC*** $C_9H_{12}N_4S_2O_2$	Brownish yellow	63	176	39.52 (39.71)	4.19 (4.41)	20.46 (20.59)
3	***5-NTBuTSC*** $C_{10}H_{14}N_4S_2O_2$	Brick red	65	190	42.31 (41.96)	4.61 (4.89)	19.35 (19.58)
4	***5-NTisoBuTSC*** $C_{10}H_{14}N_4S_2O_2$	Yellow	67	183	42.27 (41.96)	4.71 (4.89)	19.39 (19.58)
5	***5-NTdiEtTSC*** $C_{10}H_{14}N_4S_2O_2$	Brick red	69	190	42.31 (41.96)	4.61 (4.89)	19.35 (19.58)
6	***5-NT-diPrTSC*** $C_{14}H_{21}N_3S_2$	Black-brown	73	106	46.20 (45.86)	5.95 (5.73)	17.49 (17.83)
7	***5-NT-diisoBuTSC*** $C_{14}H_{22}N_4S_2O_2$	Greenish brown	58	180	49.01 (49.12)	6.52 (6.43)	16.24 (16.37)
8	***5-NT-NMCHTSC*** $C_{13}H_{18}N_4S_2O_2$	Yellow	74	190	47.71 (47.85)	5.81 (5.52)	17.44 (17.17)
9	***5-NT-CPTSC*** $C_{11}H_{14}N_4S_2O_2$	Yellow	74	190	47.71 (47.85)	5.81 (5.52)	17.44 (17.17)
10	***5-NT-CHTSC*** $C_{12}H_{16}N_4S_2O_2$	Dirty yellow	74	190	47.71 (47.85)	5.81 (5.52)	17.44 (17.17)
11	***5-NT-HMINTSC*** $C_{12}H_{16}N_4S_2O_2$	Dark brown	53	160	45.83 (46.15)	5.24 (5.13)	17.61 (17.95)

[a]For abbreviations, see Figure 2.



Scheme 1.

Chemistry

O'Sullivan reported di-methyl, di-ethyl, di-*n*-propyl, di-*n*-butyl, and methylphenyl thiocarbamylthioglycolic acid.[19] All the thioglycolic acids were prepared by the same method (Scheme 1). Cycloalkylaminothiocarbonylhydrazines were prepared by refluxing the alkaline solution of thioglycolic acid with hydrazine hydrate and their thiosemicarbazones were synthesized by refluxing the solution of cycloalkylaminothiocarbonyl hydrazines (0.003 mol) in water (10 mL) and the alcoholic solution of 5-nitrothiophene-2-carboxaldehyde (0.003 mol) at 25 °C for 3 h with continuous stirring. After cooling, the compounds were filtered and recrystallized from appropriate solvent.

The new thiosemicarbazone derivatives **1**–**11** (Fig. 2) were obtained in satisfactory yields. Melting point determination was carried out to check the purity of the compounds. Structures were confirmed by electronic, IR and ^{1}H NMR spectral studies. Analytical and spectral data (IR, electronic and ^{1}H NMR)[20] are in good agreement with the composition of thiosemicarbazones. Other analytical and physiochemical data of the compounds are presented in Table 1.

Biological Studies

In vitro activity against *E. histolytica*, *G. lamblia* and *T. vaginalis*

All the compounds were tested for in vitro activity against *E. histolytica* (strain HK-9), *G. lamblia* (strain IMSS-0989) and *T. vaginalis* (strain tv-43). *E. histolytica* and *G. lamblia* were maintained in TYIS-33 medium[21] (supplemented with bile for *G. lamblia*) at 37 °C. *T. vaginalis* parasites were maintained in PEHPS medium[22] at 37 °C. The drug potency was determined by microtiter plate method.[23] Metronidazole was used as a reference drug in all experiments. The biological test was carried out using DMSO as solvent (40 μL) in which the compounds are stable. The maximum con-

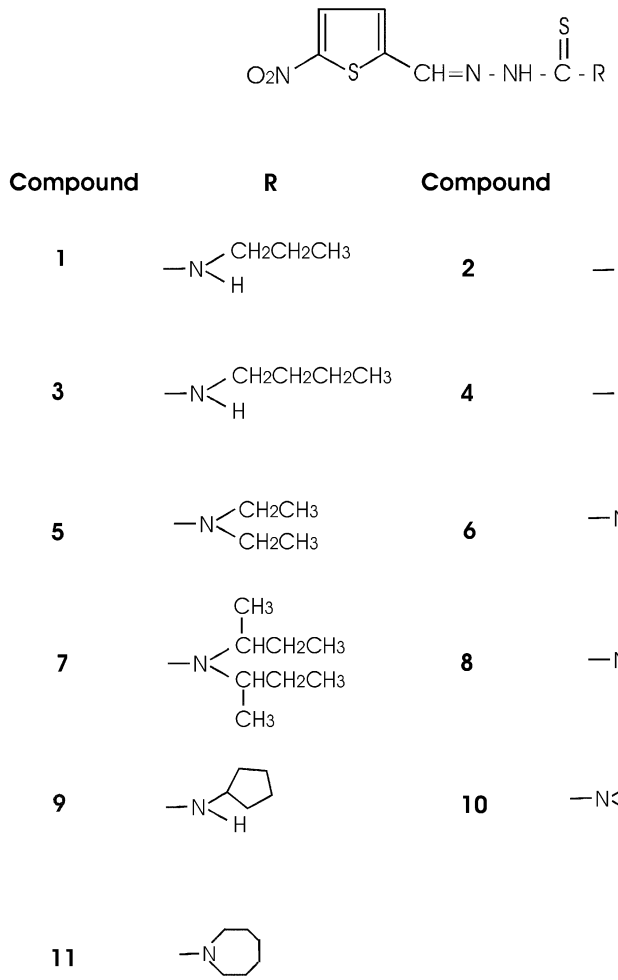


Figure 2. Structure of thiosemicarbazones. Abbreviations: (**1**) 5-nitrothiophene-2-carbaldehyde-*N*(4)propyl thiosemicarbazone: 5-NTPrTSC; (**2**) 5-nitrothiophene-2-carbaldehyde-*N*(4)isopropyl thiosemicarbazone: 5-NT-isoPrTSC; (**3**) 5-nitrothiophene-2-carbaldehyde-*N*(4)butyl thiosemicarbazone: 5-NTBuTSC; (**4**) 5-nitrothiophene-2-carbaldehyde-*N*(4)isobutyl thiosemicarbazone: 5-NTisoBuTSC; (**5**) 5-nitrothiophene-2-carbaldehyde-*N*(4,4)diethyl thiosemicarbazone: 5-NTdiEtTSC; (**6**) 5-nitrothiophene-2-carbaldehyde-*N*(4,4)dipropyl thiosemicarbazone: 5-NT-diPrTSC; (**7**) 5-nitrothiophene-2-carbaldehyde-*N*(4,4)diisobutyl thiosemicarbazone: 5-NT-diisoBuTSC; (**8**) 5-nitrothiophene-2-carbaldehyde-*N*(4,4)methylcyclohexylthiosemicarbazone: 5-NT-MCHTSC; (**9**) 5-nitrothiophene-2-carbaldehyde-*N*(4)cyclopentyl thiosemicarbazone: 5-NT-CPTSC; (**10**) 5-nitrothiophene-2-carbaldehyde-*N*(4)cyclohexyl thiosemicarbazone: 5-NT-CHTSC; (**11**) 5-nitrothiophene-2-carbaldehyde-*N*(4)hexamethyleneiminethiosemicarbazone: 5-NT-HMINTSC.

centration of DMSO in the test did not exceed 0.1%, at which level no inhibition of amoebal growth occurred.[24] In vitro antiprotozoal activities of all the thiosemicarbazones are listed in Table 2. 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. At least three experiments were performed for each compound tested. Metronidazole had a 50% inhibition concentration (IC_{50}) of 2.10 μM in our experiment, which lies within the range of IC_{50} value of metronidazole (2.92 μM) as reported previously.[25,26]

Even though, most of the compound studied did not reach to our expectations, conclusion that could be drawn were: (i) replacement of the terminal group at N^4 position may affect the activity; (ii) compounds which were found very effective against *E. histolytica* were almost ineffective against *G. lamblia*. Cerecetto et al.[14] synthesized semicarbazones of 5-nitrothiophene-2-carboxaldehyde by using amines like butyl, hexyl, morpholine, 1-methylpiperizine, 2-methoxyethyl, 2-phenylethyl and 2-(3,4-dimethoxyphenyl)ethyl amine. These results concluded that semicarbazone derivatives containing a ring in their aminic portion showed very poor antitrypanosomal activity. In comparison to butyl and hexyl semicarbazones of 5-nitrothiophene-2-carboxaldehyde showed that these compounds were found less active whereas compound **3** and **10** (Table 2) showed better Trichomonial and antiamoebic activity, respectively. Thiosemicarbazones of 5-nitrothiophene-2-carboxalehyde showed better activity than the semicarbazone on protozoal diseases. It was noted that antiparasitic activity was limited to those compounds in which the alkylidene group is attached to the 2-position, rather than the 3- or 4-position, of the heterocyclic ring, and also to those in which a thiocarbonyl, rather than a carbonyl group, is present.[27] Studies of the toxicity of

Table 2. In vitro activities of thiosemicarbazones against *E. histolytica* (HK-9), *T. vaginalis* (tv-43) and *G. lamblia* (IMSS-0989)

Compd	Antiamoebic IC_{50} (μM)	Antitrichomonal IC_{50} (μM)	Antigiardial IC_{50} (μM)
1	15.38±1.94	21.03±3.16	57.26±7.28
2	9.60±1.07	5.94±0.89	26.19±3.72
3	12.17±0.98	1.49±0.31	17.19±2.63
4	13.50±2.15	6.31±1.14	33.33±4.87
5	10.10±1.46	6.01±0.85	8.03±1.34
6	6.40±0.91	4.06±0.65	8.20±1.19
7	11.63±2.05	4.44±3.07	12.01±1.86
8	8.30±1.26	18.30±3.07	11.72±1.42
9	2.86±0.45	3.97±0.59	32.99±4.07
10	2.41±0.51	10.70±1.34	13.90±2.15
11	1.71±0.38	8.78±1.16	8.47±1.24
MNZ	2.10±0.33	1.92±0.28	1.16±0.21

compound **3** and **11** towards mammalian cells are currently underway, as well as in vivo studies on trichomonocidal and amoebicidal activity.

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20. **1**. λ_{max}/cm^{-1}: 24,752, 35,842, 40,816, 48,780; ν_{max}/cm^{-1}: 3322 (NH), 1556 (C=N), 1135 (C–N), 1033 (C=S); ^1H NMR ($(CD_3)_2SO$)/ppm: 8.04 (1H, s, –CH=N), 11.40 (1H, s, –NH), 8.25 (1H, t, –NH), 4.20 (4H, m, $–CH_2$), 2.21 (3H, t, $–CH_3$), 7.03–8.12 (2H, m, aryl). **2**. λ_{max}/cm^{-1}: 25,000, 36,101, 40,322, 48,309; ν_{max}/cm^{-1}: 3428 (NH), 1600 (C=N), 1093 (C–N), 1035 (C=S); ^1H NMR ($(CD_3)_2SO$)/ppm: 8.06 (1H, s, –CH=N), 10.62 (1H, s, –NH), 8.47(1H, d, –NH), 2.95 (6H, d, $–CH_3$), 6.93–7.82 (2H, m, aryl). **3**. λ_{max}/cm^{-1}: 24,752, 36,496, 38,610, 48,309; ν_{max}/cm^{-1}: 3434 (NH), 1590 (C=N), 1095 (C–N), 1040 (C=S); ^1H NMR ($(CD_3)_2SO$)/ppm: 8.39 (1H, s, –CH=N), 10.96 (1H, s, –NH), 8.51 (1H, t, –NH), 4.12 (6H, m, $–CH_2$), 2.19 (3H, t, $–CH_3$), 7.06–8.01 (2H, m, aryl). **4**. λ_{max}/cm^{-1}: 25,470, 36,232, 38,461, 48,544; ν_{max}/cm^{-1}: 3460 (NH), 1595 (C=N), 1106 (C–N), 1052 (C=S), ^1H NMR ($(CD_3)_2SO$)/ppm: 8.09 (1H, s, –CH=N), 11.23 (1H, s, –NH), 8.21(1H, d, –NH), 4.46 (2H, m, $–CH_2$), 1.99 (6H, m, $–CH_3$), 7.23–8.04 (2H, m, aryl). **5**. λ_{max}/cm^{-1}: 24,752, 36,496, 38,712, 45,045; IR: ν_{max}/cm^{-1}: 3384 (NH), 1585 (C=N), 1106 (C–N), 1036 (C=S); ^1H NMR ($(CD_3)_2SO$)/ppm: 8.39 (1H, s, –CH=N), 10.02 (1H, s, –NH), 2.53 (6H, m, $–CH_3$), 4.23 (4H, m, $–CH_2$), 7.40–8.12 (2H, m, aryl). **6**. λ_{max}/cm^{-1}: 24,630, 35,211, 47,847; ν_{max}/cm^{-1}: 3494 (NH), 1492 (C=N), 1121 (C–N), 1050 (C=S); ^1H NMR ($(CD_3)_2SO$)/ppm: 8.29 (1H, s, –CH=N), 10.43 (1H, s, –NH), 4.30 (8H, m, $–CH_2$), 2.19 (6H, t, $–CH_3$), 7.01–7.92 (2H, m, aryl). **7**. λ_{max}/cm^{-1}: 24,938, 37,879, 48,309; ν_{max}/cm^{-1}: 3422 (NH), 1630 (C=N), 1117 (C–N), 1037 (C=S); ^1H NMR ($(CD_3)_2SO$)/ppm: 8.45 (1H, s, –CH=N), 9.98 (1H, s, –NH), 3.98 (4H, m, $–CH_2$), 1.99 (12H, m, $–CH_3$), 6.89–7.53 (2H, m, aryl). **8**. λ_{max}/cm^{-1}: 24,630, 34,965, 48,312; ν_{max}/cm^{-1}: 3469 (NH), 1570 (C=N), 1139 (C–N), 1087 (C=S); ^1H NMR ($(CD_3)_2SO$)/ppm: 8.34 (1H, s, –CH=N), 11.14 (1H, s, –NH), 4.53 (10H, m, $–CH_2$), 2.56 (3H, s, $–CH_3$), 7.23–8.04 (2H, m, aryl). 9.λ_{max}/cm^{-1}: 24,635, 35,461, 40,123, 49,020; IR: ν_{max}/cm^{-1}: 3456 (NH), 1664 (C=N), 1120 (C–N), 1039 (C=S); ^1H NMR ($(CD_3)_2SO$)/ppm: 8.44 (1H, s, –CH=N), 10.69 (1H, s, –NH), 8.76 (1H, d, –NH), 4.23 (8H, m, $–CH_2$), 7.07–7.87 (2H, m, aryl). **10**. λ_{max}/cm^{-1}: 24,755, 35,714, 40,112, 48,913; IR: ν_{max}/cm^{-1}: 3472 (NH), 1532 (C=N), 1108 (C–N), 1034 (C=S); ^1H NMR ($(CD_3)_2SO$)/ppm: 8.21 (1H, s, –CH=N), 10.05 (1H, s, –NH), 8.65 (1H, d, –NH), 4.53 (10H, m, $–CH_2$), 6.97–7.54 (2H, m, aryl). **11**. λ_{max}/cm^{-1}: 24,630, 36,101, 38,315, 48,780; ν_{max}/cm^{-1}: 3402 (NH), 1583 (C=N), 1090 (C–N), 1035 (C=S); ^1H NMR ($(CD_3)_2SO$)/ppm: 8.23 (1H, s, –CH=N), 9.81 (1H, s, –NH), 4.39 (12H, m, $–CH_2$), 7.13–7.79 (2H, m, aryl).
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