Molecules **2007**, *12*, 622-633

molecules

ISSN 1420-3049

http://www.mdpi.org

Full Paper

Synthesis and *in vitro* Evaluation of New Benzothiazole Derivatives as Schistosomicidal Agents†

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† Part of this work was presented as a poster (No. 37) at the 2nd Mediterranean Organic Chemistry Meeting, Almeria, Spain, March 2003.

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Received: 9 November 2006; in revised form: 21 March 2007 / Accepted: 23 March 2007 / Published: 26 March 2007

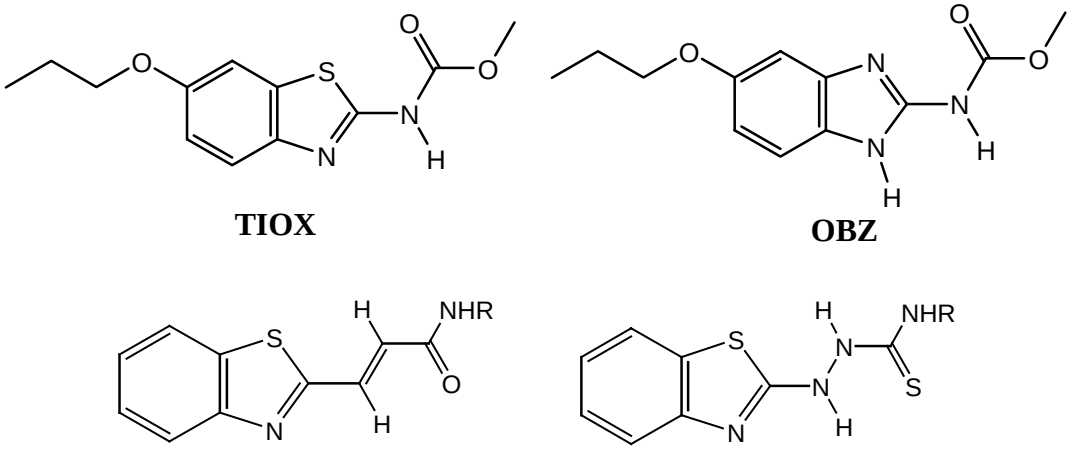
Abstract: A series of benzothiazol-2-yl-dithiocarbamates **3a-d** along with their copper complexes **4a-c** were synthesized via the reaction of suitable alkyl, aralkyl or heteroaryl halides with the sodium salt of benzothiazol-2-yl-dithiocarbamic acid, followed by complexation with copper sulphate. *N*-(4-Acetyl-5-aryl-4,5-dihydro-1,3,4-thiadiazol-2-yl)-N-benzothiazol-2-yl-acetamides **7a-c** were synthesized by cyclization of the appropriate thiosemicarbazones **6a-c** in acetic anhydride. Selected compounds were screened for *in vitro* schistosomicidal activity against *Schistosoma mansoni* at three different dosage levels (10, 50 and 100 µg/ mL). Three of these products, **4a-c**, showed schistosomicidal activity similar to praziquantel, with 100% worm mortality at 10 µg/mL. These compounds would constitute a new class of potent schistosomicidal agents.

Keywords: Benzothiazole, dithiocarbamate, thiadiazole, thiosemicarbazones, schistosomicidal agents.

Introduction

Anthelmintics exert their chemotherapeutic effect by interfering with some biochemical or physiological process(es) essential for the survival of the parasite in the host [1]. Several substituted benzimidazoles [2,3] and benzothiazoles [2-5] have been identified as potent anthelmintic drugs. The benzothiazole anthelimintic methyl 6-propoxybenzothiazole-2-carbamate (tioxidazole, TIOX, Figure 1), was reported to exert its chemotherapeutic action on *Hymenolepis diminuta* by decreasing the ability of the worm to absorb and metabolize exogenous glucose [3]. The similarity of biochemical and physiological effects of benzothiazole and benzimidazole anthelmintics on tapeworms suggested a common mode of action and molecular modeling studies have revealed that both benzothiazole and benzimidazole are electronically similar [3]**.**

Figure 1

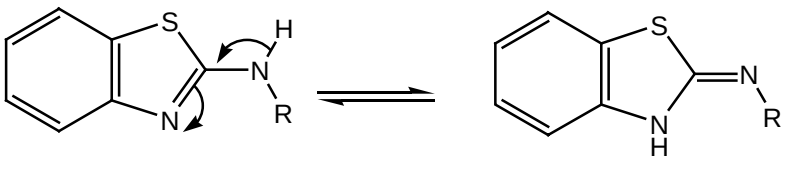


A: R= N=CHC$_6$H$_4$-Cl(4)
B: R= NHCSNHC$_6$H$_5$

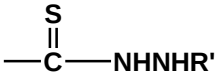
C: R= C$_6$H$_4$Br(4)

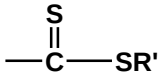
Two possible explanations have been put forward to explain why TIOX is a less potent anthelmintic compared to methyl 6-propoxybenzimidazole-2-carbamate (oxibendazole, OBZ, Figure 1). One explanation is that TIOX, the benzothiazole analogue, lacks the acidic hydrogen found on the extra nitrogen atom in the benzimidazole anthelmintics. Another explanation was that TIOX is more susceptible to metabolic processes than OBZ, and thus reaches its intended site of action at lower concentrations. Furthermore, several benzothiazole analogs (**A, B**, Figure 1) were reported to possess potent schistosomicidial activity [6]. Recently, we noted that compound **C** (Figure 1), a benzothiazol-2-ylthiosemicarbazide derivative, exhibited very high antischistosomal activity against *S. mansoni* (91.7% reduction of worm numbers at 20 mg/Kg) [7]. In view of this information, two series of benzothiazole derivatives were prepared and evaluated for *in vitro* schistosomicidal activity. These compounds were designed to possess an NH group at the 2 position of the benzothiazole ring, thus allowing for tautomeric structures **D** and **F** (Figure 2). In addition, different moieties (thiosemicarbazide or dithiocarbamic acid) were attached to the benzothiazole ring, along with aromatic, heterocyclic or acyclic groups, in order to examine the effect of such structure modifications on the biological activity.

Figure 2



D **F**

R= 

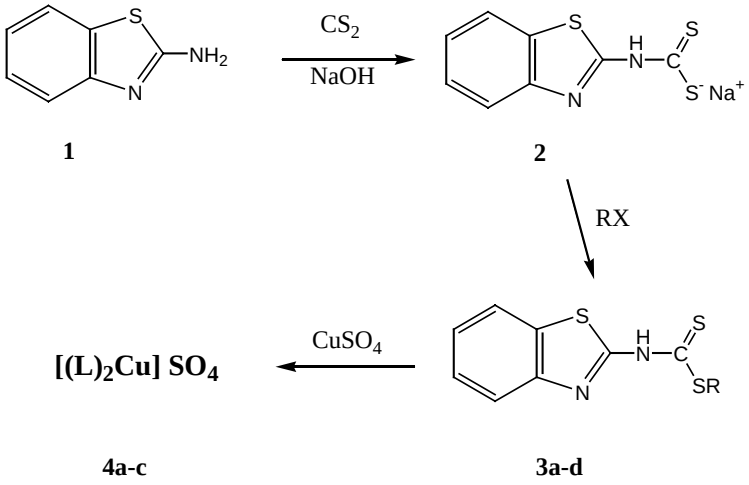
= 

Results and Discussion

Synthesis

The synthesis of the new compounds was achieved following the linear pathway strategy summarized in Schemes 1 and 2.

Scheme 1



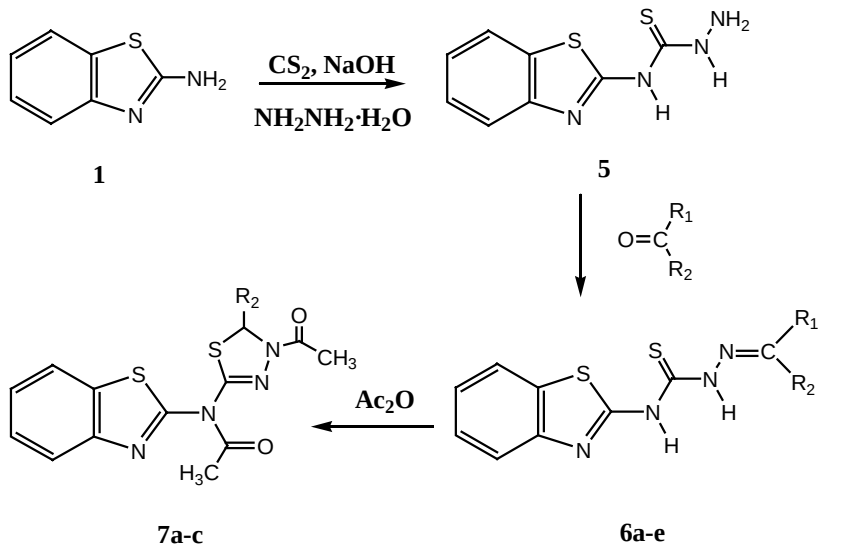
	a	**b**	**c**
L	**3a**	**3b**	**3d**

	a	**b**	**c**	**d**
R	**C_3H_7**	**$CH_2C_6H_5$**	**CH_2COOEt**	

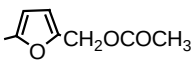
The key intermediate, sodium dithiocarbamate salt **2**, was prepared according to the reported procedure [8], using the reaction of commercially available 2-aminobenzothiazole and carbon disulfide in an alkaline medium. The dithiocarbamate esters **3a-d** were obtained through alkylation of compound **2** using different alkyl, aralkyl or heteroaryl halides. Since several metal complex derivatives were reported to display increased drug pharmacological potency [9,10], copper complexes **4a-c** of compounds **3a, b, d** were prepared using $CuSO_4 \cdot 5H_2O$ in boiling aqueous ethanol solution. Reaction of 4-(Benzothiazol-2-yl)thiosemicarbazide **5** [11] with various carbonyl compounds gave the

corresponding thiosemicarbazones **6a-e**. Cyclization of **6a-c** in acetic anhydride afforded the corresponding N-(4-acetyl-5-aryl-4,5-dihydro-1,3,4-thiadiazol-2-yl)-N-benzothiazol-2-yl-acetamides **7a-c**. The structures of the new compounds were confirmed on the basis of their spectral and microanalytical data.

Scheme 2



1 **5** **6a-e** **7a-c**

	a	b	c	d	e
R_1	H	H	H	H	$(CH_2)_4$
R_2	4-$OCH_3C_6H_4$	4-BrC_6H_4	4-$NO_2C_6H_4$		

Schistosomicidal Activity

In the control of schistosomiasis, the use of safe and effective drugs will remain the main control tool until a successful vaccine is produced. Praziquantel (PQZ) is the only antibilharzial drug effective against all the schistosomes pathogenic to man [12]. Although PQZ has minimal side effects, its widespread use in control of schistosomiasis faces some problems. Clinically, reduced cure rates and treatment failure have been reported after PQZ use in patients in developing countries [13-15]. For these reasons, the search for new drugs with antischistosomal activity is justifiable. In this study selected compounds **2-7** were tested, *in vitro*, against *Schistosoma mansoni* adult worms at three different doses (10, 50 and 100 ug/mL) [16]. The results obtained (Table 1) revealed that the intermediates **2** exhibited weak activity, while its corresponding esters **3a, c** and **d** showed moderate potency. Interestingly, the respective copper complexes **4a-c** possessed the highest activity (100% mortality at10 μg/mL). On the other hand, the activity of compound **5** was proportional to its concentration, while the corresponding thiosemicarbazones **6a-e** showed a dramatic decrease in activity (compounds **6a,c** were totally inactive). Finally, thiadiazole derivatives **7a-c** were moderately active. In general, it could therefore be concluded that a dithiocarbamate link is desirable for better

activity. In addition, copper complexes **4a-c** constituted very promising antischistosomal candidates and further *in vivo* evaluation of these compounds is currently in progress.

Table 1: Percentage *Schistosoma mansoni* worm mortality under different concentrations of praziquantel (PZQ) and the newly synthesized compounds.

Compound	Concentration		
	100 µg	**50 µg**	**10 µg**
	Percentage mortality of worms		
2	100%	0%	0%
3a	90%	80%	20%
3c	100%	80%	50%
3d	50%	40%	10%
4a	100%	100%	100%
4b	100%	100%	100%
4c	100%	100%	100%
5	100%	50%	0%
6a	0%	0%	0%
6b	10%	12.5%	0%
6c	0%	0%	0%
6d	80%	70%	0%
6e	100%	8.3%	0%
7a	100%	35.7%	0%
7b	100%	58%	0%
7c	100%	40%	0%
Positive control (PQZ)	100%	100%	100%
Negative control (DMSO)	0%	0%	0%

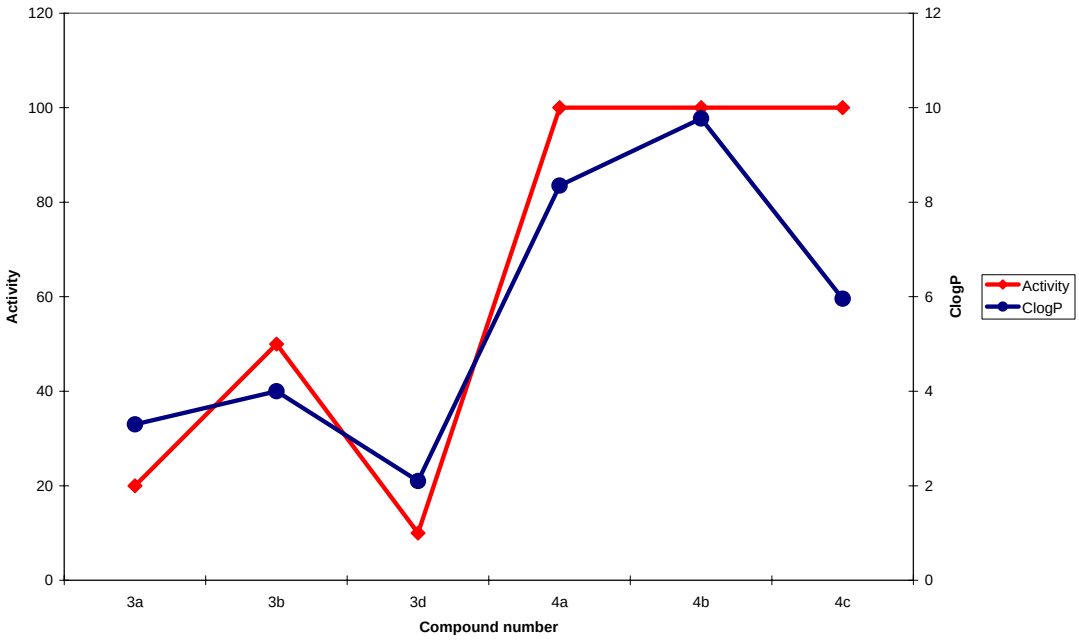
ClogP study

In an attempt to rationalize the optimum activity of the metal-ligand complexes **4a-c** compared to its free ligands **3a, b** and **d**, a study of the corresponding activity-lipophilicity relationships was performed. ClogP values of the aforementioned compounds were calculated (see the experimental section) using the ACD/logP software. Plotting of the caculated ClogP values of the compounds against their activity (Figure 3), revealed a strong correlation between lipophilicity and schistosomicidal activity for all studied compounds (correlation coefficient = 0.91), except for compound **4c**, that showed a slight deviation between the two values (ClogP = 5.96, activity 100% at 10 µg/mL).

Acute toxicity

Compounds **4a-c,** the most active analogs, was further evaluated for their oral toxicity in male mice using a reported method [17,18]. The results obtained revealed that none of the the test compounds were toxic and that they were well tolerated by the experimental animals up to 250 mg/kg (zero % of mortality).

Figure 3



Experimental

General

Melting points were determined in open glass capillaries and are uncorrected. Infrared spectra (IR) were measured, using KBr discs, on a Perkin-Elmer 1430 Infrared spectrophotometer. ^{1}H-NMR spectra were recorded on Jeol JNM-LA400 FT-NMR (400 MHz) or JNM-LA500 (500 MHz) systems; chemical shift (δ) are reported in ppm using TMS as an internal reference and coupling constant (*J*) in Hz. Mass spectra were run on a Finnigan model SSQ/7000 mass spectrometer (70ev). Elemental analyses were run using a Perkin-Elmer RE 2400 CHNS analyzer. Copper analysis was performed on a Perkin-Elmer 2380 atomic absorption spectrophotometer. Reactions were monitored by thin-layer chromatography (TLC) on silica gel (60 GF 353; Merck). Spots were visualized by exposure to iodine vapour or UV-lamp at 254 n.m.

Sodium benzothiazol-2-yldithiocarbamate (**2**)

A stirred solution of 2-aminobenzothiazole (3 g, 20 mmol) in dimethylformamide (10 mL) was treated with sodium hydroxide (0.8 g, 20 mmol). To this mixture carbon disulphide (1.2 mL, 20 mmol) was added with stirring over a period of 15 minute. Stirring was continued until all the sodium hydroxide dissolved. Finally, excess chloroform was added and the yellow sodium salt obtained was filtered off, washed with chloroform and dried. Yield: 2.5 g (50.4%), m.p. 298-300°C (10% DMF/ $CHCl_3$). Anal.: ($C_8H_5N_2S_3Na$) Calc.: C 38.69, H 2.03, N 11.28. Found: C 38.80, H 2.0, N 11.27.

Alkyl, aralkyl or heteroaryl (benzothiazol-2-yl) dithiocarbamates **3a-d**

To a stirred hot solution of **2** (2.48 g, 10 mmol) in EtOH (10 mL) the appropriate alkyl, aralkyl or heteroaryl halide (12 mmol) was gradually added. The reaction mixture was heated under reflux for 30

min. The solvent was evaporated under vacuum and the residual mixture was stirred with excess ice-cold water. The precipitate that separated was filtered, washed with ice-cold water (3 x 10 mL) and crystallized from aqueous ethanol.

Propyl benzothiazol-2-yldithiocarbamate (**3a**). Propyl iodide (2.04 g, 12 mmol) was used. Yield: 1.69 g (63%); yellow crystals; m.p: 132-133°C; IR (cm^{-1}): 3438 (NH), 1660 (C=N), 1567 (C=S); ^{1}H -NMR ($CDCl_3$): 1.04 (t, 3H, *J* = 6.9, CH_3), 1.77 (sextet, 2H, *J* = 7.6, CH_2), 3.29 (t, 2H, *J* = 6.9, SCH_2), 7.35 (t, 1H, *J* = 7.65, benzothiazole-C_6-H), 7.46 (t, 1H, *J* = 7.65, benzothiazole-C_5-H), 7.75 (d, 1H, *J* = 7.65, benzothiazole-C_7-H), 7.77 (d, 1H, *J* = 7.65, benzothiazole-C_4-H), 11.55 (br. s, 1H, NH); Anal. Calc. for $C_{11}H_{12}N_2S_3$: C 49.22, H 4.51, N 10.44. Found: C 49.31, H 4.7, N 10.30; ClogP = 3.3.

Benzyl benzothiazol-2-yldithiocarbamate (**3b**). Benzyl chloride (1.52 g, 12 mmol) was used. Yield: 1.61g (51%); yellow crystals; m.p: 140-141°C; IR (cm^{-1}): 3437 (NH), 1661 (C=N), 1567 (C=S); ^{1}H-NMR ($CDCl_3$): 4.58 (s, 2H, SCH_2), 7.26-7.31(m, 3H, Ar- $C_{3,4,5}$-H), 7.32-7.36 (m, 2H, benzothiazole-$C_{5,6}$-H), 7.39 (d, 2H, *J* = 7.65, Ar- $C_{2,6}$-H), 7.61(d, 1H, *J* = 7.65, benzothiazole- C_7-H), 7.74 (d, 1H, *J* = 7.65, benzothiazole-C_4-H), 12.58 (br. s, 1H, NH); MS: (% abundance); 317(3.1) M+1, 316 (4.6) $M^+[C_{15}H_{12}N_2S_3]$, 315 (18.8) M-1, 193 (21.6) [M-$SCH_2C_6H_5$], 91 (100) [$C_6H_5CH_2$]; ClogP = 4.0.

Ethyl [(benzothiazol-2-yl)thiocarbamoyl]sulfanylacetate (**3c**). Ethyl chloroacetate (1.74 g, 12 mmol) was used. Yield: 2.18 g (70%); yellow crystals; m.p: 149-150°C; ^{1}H-NMR ($CDCl_3$): 1.39 (t, 3H, *J* = 6.9, CH_3), 4.62 (q, 2H, *J* = 6.1, CO_2CH_2), 4.65 (s, 2H, SCH_2), 7.33 (t, 1H, *J* = 7.65, benzothiazole-C_6-H), 7.44 (t, 1H, *J* = 7.65, benzothiazole-C_5-H), 7.81 (d, 1H, *J* = 7.65, benzothiazole-C_7-H), 7.84 (d, 1H, *J* = 8.4, benzothiazole-C_4-H), 11.92 (br.s, 1H, NH); Anal. Calc. for $C_{12}H_{12}N_2O_2S_3$: C 46.13, H 3.87, Found: C 46.36, H 3.93.

3-Nitropyrid-2-yldithiocarbamate-2-benzothiazole (**3d**). 2-Chloro-3-nitropyridine (1.9 g, 12 mmol) was used. Yield: 1.97 g (57%); orange crystals; m.p: 156-157°C; IR (cm^{-1}): 3437 (NH), 1660 (C=N), 1568 (C=S), 1517, 1349 (NO_2); ^{1}H-NMR (DMSO-d_6): 7.30 (t, 1H, *J* =7.65, benzothiazole-C_6-H), 7.43 (t, 1H, *J* = 7.65, benzothiazole-C_5-H), 7.52(dd, 1H, *J* = 4.6, 8.4, pyridine-C_5-H), 7.62 (d, 1H, *J* = 6.9, benzothiazole-C_7-H), 7.93 (d, 1H, *J* = 7.6, benzothiazole-C_4-H), 8.65(d, 1H, *J* = 8.4, pyridine-C_4-H) 8.69(d, 1H, *J* = 4.6, pyridine-C_6-H); Anal. Calc. for $C_{13}H_8N_4O_2S_3$: C 44.81, H 2.31, Found: C 44.60, H 2.31; ClogP = 2.1

Copper complexes of ligands **3a, b** *and* **d** (**4a-c**)

To a hot stirred solution of $CuSO_4 \cdot 5H_2O$ (2.49 g, 10 mmol) in aqueous ethanol (1:1, 10 mL) a solution of the corresponding dithiocarbamate esters **3a, b,** or **d** (20 mmol) in hot ethanol (20 mL), was added. The mixture was refluxed for 1 h on a boiling water bath and the reaction mixture was set aside to attain room temperature. The solid obtained was filtered, washed with ethanol, dried and crystallized from 3:7 aqueous ethanol.

Propyl benzothiazol-2-yldithiocarbamate copper complex (**4a**). Compound **3a** (5.36 g, 20 mmol) was used. Yield: 2.7 g (18.6%); orange brown solid; m.p.: 126-128°C (dec.); IR (cm^{-1}): 3437 (NH), 1661

(C=N), 1550 (C=S), 387 (Cu-N), 293 (Cu-S); ^{1}H-NMR (DMSO-d_6): 0.84-0.94 (m, 5H, CH_2CH_3), 4.19 (t, 2H, *J* = 6.9, SCH_2), 7.23 (t, 1H, *J* = 7.65, benzothiazole-C_6-H), 7.36 (t, 1H, *J* = 7.65, benzothiazole-C_5-H), 7.65 (d, 1H, *J* = 7.6, benzothiazole-C_7-H), 7.9 (d, 1H, *J* = 8.4, benzothiazole-C_4-H), 12.01 (s, 1H, NH); MS: (% abundance); 267(19.6) [$C_{11}H_{11}N_2S_3$], 266 (22.7) [$C_{11}H_{10}N_2S_3$], 209 (52.1) [$C_9H_9N_2S_2$], 193 (47.6) [$C_8H_5N_2S_2$], 176 (100) [$C_9H_8N_2S$], 65 (3.4) [Cu^{65}], 63 (9.2) [Cu^{63}]; ClogP = 8.35.

Benzyl benzothiazol-2-yldithiocarbamate copper complex (**4b**). Compound **3b** (6.32 g, 20 mmol) was used. Yield: 3.2g (20%); m.p.100-102°C (dec.); IR (cm^{-1}): 3413 (NH), 1661 (C=N), 1549 (C=S), 387 (Cu-N), 293 (Cu-S); ^{1}H-NMR (DMSO- d_6): 4.44 (s, 2H, CH_2), 7.15-7.26 (m, 5H, Ar-H), 7.28-7.32 (m, 2H, benzothiazole-$C_{5,6}$-H), 7.37 (d, 1H, *J* = 6.9, benzothiazole- C_7-H), 7.76 (d, 1H, *J* = 7.65, benzothiazole-C_4-H); Anal. Calc. for $C_{30}H_{24}CuN_4O_4S_7$: N 7.07, Cu 8.02. Found: N 7.16, Cu 8.03; ClogP = 9.77.

3-Nitropyrid-2-yldithiocarbamate -2-benzothiazole copper complex (**4c**). Compound **3d** (6.96g, 20 mmol) was used. Yield 5g (30 %); m.p. 166-168 °C (dec.); IR (cm^{-1}): 3411 (NH), 1660 (C=N), 1550 (C=S), 1517, 1352 (NO_2), 387 (Cu-N), 293 (Cu-S), ^{1}H-NMR 6.98-8.06 (m, 5H, benzothiazole and pyridine-C_5-H), 8.66 (d, 1H, *J* = 8.4, pyridine-C_4-H), 8.7 (d, 1H, *J* = 3.8, pyridine-C_6-H), 9.0 (s, 1H, NH); Anal. Calc. for $C_{26}H_{16}CuN_8O_8S_7$: N 13.08, Cu 7.42. Found: N 13.1, Cu 7.4; ClogP = 5.96.

4-(Benzothiazol-2-yl)-1-aryl, cycloalkyl or heteroaryl thiosemicarbazones **6a-e**

A mixture of 4-(benzothiazol-2-yl)thiosemicarbazide **5** [11] (2.24 g, 10 mmol), the appropriate aldehyde or ketone (12 mmol) in absolute ethanol (15 mL) containing a few drops of glacial acetic acid were heated under reflux for 1 h, except for cyclopentanone (8 h). The precipitate formed after cooling was collected by filtration, dried and crystallized from ethanol.

4-(Benzothiazol-2-yl)-1-(4-methoxybenzylidene)thiosemicarbazide (**6a**). 4-Methoxybenzaldehyde (1.63 g, 12 mmol) was used. Yield: 1.68 g (49%); pale yellow crystals; m.p: 188-190°C; IR (cm^{-1}): 3151 (NH), 1601 (C=N), 1560 (C=S), 1235 (C-O-C); ^{1}H-NMR (DMSO-d_6): 3.79 (s, 3H, OCH_3), 6.99 (d, 2H, *J* = 8.4, Ar-$C_{3,5}$-H), 7.29 (t, 1H, *J* = 7.65, benzothiazole-C_6-H), 7.41(t, 1H, *J* = 7.65, benzothiazole-C_5-H) 7.96 (d, 1H, *J* = 7.65, benzothiazole-C_7-H), 7.86 (d, 2H, *J* = 6.9, Ar-$C_{2,6}$-H), 7.94(d, 1H, *J* = 7.65, benzothiazole-C_4-H), 8.15 (s, 1H, CH=N), 11.53, 12.4 (two br.s, each 1H, two NH); Anal. Calc. for $C_{16}H_{14}N_4OS_2 \cdot ¼\ H_2O$: C 55.39, H 4.21, N 16.15, Found: C 55.31, H 3.69, N 15.99.

4-(Benzothiazol-2-yl)-1-(4-bromobenzylidene)thiosemicarbazide (**6b**). 4-Bromobenzaldehyde (2.22 g, 12 mmol) was used. Yield: 1.80 g (46%); yellow crystals; m.p: 216-217°C; IR (cm^{-1}): 3433, 3328 (NH), 1594 (C=N), 1476 (C=S); ^{1}H-NMR (DMSO-d_6): 7.29 (t, 1H, *J* = 7.65, benzothiazole-C_6-H), 7.14 (t, 1H, *J* = 7.65, benzothiazole-C_5-H), 7.63 (d, 2H, *J* = 8, Ar-$C_{2,6}$-H), 7.69 (d, 1H, *J* = 6.9, benzothiazole-C_7-H), 7.95-7.86 (m, 3H, benzothiazole-C_4-H and Ar-C-$_{3,5}$-H), 8.17(s, 1H, CH=N), 12.48, 12.89 (two br.s, each 1H, two NH); Anal. Calc. for $C_{15}H_{11}BrN_4S_2 \cdot ¼\ H_2O$: C 45.52, H 2.93, N 14.15. Found: C 45.17, H 2.48, N 14.50

4-(Benzothiazol-2-yl)-1-(4-nitrobenzylidene)thiosemicarbazide (**6c**). 4-Nitrobenzaldehyde (1.81 g, 12 mmol) was used. Yield: 1.9 g (53%); yellow crystals; m.p: 220-222°C; IR (cm^{-1}): 3439, 3306 (NH), 1643 (C=N), 1593 (C=S), 1551, 1381 (NO_2); ^{1}H-NMR (DMSO-d_6): 7.30 (t, 1H, *J* = 7.65, benzothiazole-C_6-H), 7.43 (t, 1H, *J* = 7.65, benzothiazole-C_5-H), 7.76-7.70 (m, 2H, Ar-$C_{2,6}$-H), 7.94 (d, 1H, *J* = 7.65, benzothiazole-C_7-H), 8.3-8.2 (m, 2H, CH=N and benzothiazole-C_4-H), 8.27 (d, 2H, *J* = 8.4, Ar-$C_{3,5}$-H), 12.64, 12.90 (two s, each 1H, two NH); Anal. Calc. for $C_{15}H_{11}N_5O_2S_2$·¼ H_2O: N 19.35. Found: N 18.96.

5-(Benzothiazol-2-ylthiocarbamoylhydrazonomethyl)-2-furylmethyl acetate (**6d**). 5-Acetoxymethyl-2-furanaldehyde (2.02 g, 12 mmol) was used. Yield: 1.46 g (44%); pale yellow solid; m.p: 184-186°C; IR (cm^{-1}): 3430, 3259 (NH), 1787 (C=O), 1643 (C=N), 1446 (C=S), 1276 (C-O-C); ^{1}H-NMR (DMSO-d_6): 2.06 (s, 3H, CH_3), 5.1 (s, 2H, CH_2), 6.67 (d, 1H, *J* = 3.05, furan-C_3-H), 7.06 (d, 1H, *J* = 3.00, furan-C_4-H), 7.28 (t, 1H, *J* = 7.65, benzothiazole-C_6-H), 7.40 (t, 1H, *J* =7.65, benzothiazole-C_5-H), 7.16 (d, 1H, *J* = 8.3, benzothiazole-C_7-H), 7.91(d, 1H, *J* = 6.9, benzothiazole-C_4-H), 8.06 (s, 1H, CH=N), 12.17, 12.44 (two s, each 1H, two NH); Anal. Calc. for $C_{16}H_{14}N_4O_3S_2$·½ H_2O: N 14.61, S 16.73. Found: N 15.00, S 16.45

4-(Benzothiazol-2-yl)-1-(cyclopentylidene)thiosemicarbazide (**6e**). Cyclopentanone (1.00 g, 12 mmol) was used. Yield: 1.19 g (41%); pale yellow crystals; m.p: 198-200°C; IR (cm^{-1}): 3432, 3145 (NH), 1684 (C=N), 1524 (C=S); ^{1}H-NMR (DMSO-d_6): 1.67-1.80 (m, 4H, cyclopentylidene-$C_{3,4}$-H), 2.41(dd, 4H, *J* = 13, 6.1 Hz, cyclopentylidene-$C_{2,5}$-H), 7.26 (t, 1H, *J* = 7.65, benzothiazole-C_6-H), 7.38 (t, 1H, *J* = 7.65, benzothiazole-C_5-H), 7.63 (d, 1H, *J* = 7.6, benzothiazole-C_7-H), 7.91 (d, 1H, *J* = 6.9, benzothiazole-C_4-H), 11.20, 11.27 (two s, each 1H, two NH); Anal. Calc. for $C_{13}H_{14}N_4S_2$·¼ H_2O: C 52.94, H 4.96, N 19.00. Found: C 53.05, H 4.86, N 18.89

N-Benzothiazol-2-yl-N-(4-acetyl-5-aryl-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamides **7a-c**

The appropriate thiosemicarbazone **6a-c** (10 mmol) in acetic anhydride (3 mL) was heated in a water bath for 5h. The reaction mixture was poured into ice water, extracted with $CHCl_3$ (3 x 10 mL), then dried over anhydrous sodium sulfate. After filtration, the precipitate formed after addition of petroleum ether (60-80ºC) was collected by filtration and crystallized from $CHCl_3$/petroleum ether (10:1).

N-Benzothiazol-2-yl-N-[4-acetyl-5-(4-methoxyphenyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]acetamide (**7a**). Thiosemicarbazone **6a** (3.42 g, 10 mmol) was used. Yield: 1.50 g (35%); yellow solid; m.p:170-172°C; IR (cm^{-1}): 1712, 1699 (C=O), 1691 (C=N), 1606 (C=S), 1532, 1328 (C-O-C); ^{1}H-NMR (DMSO-d_6): 2.19, 2.41 (two s, each 3H, two $COCH_3$), 3.77 (s, 3H, OCH_3), 7.01 (d, 2H, *J* = 8.45, Ar-$C_{3,5}$-H), 7.32 (s, 1H, thiadiazoline-C_5-H), 7.38 (t,1H, *J* =7.65, benzothiazole-C_6-H), 7.50 (t, 1H, *J* = 7.65, benzothiazole-C_5-H), 7.68 (d, 2H, *J* = 8.4, Ar-$C_{2,6}$-H), 7.86 (d, 1H, *J* = 7.65 Hz, benzothiazole-C_7-H), 8.03 (d, 1H, *J* = 8.4, benzothiazole-C_4-H); MS: (% abundance); 426 (17.33) M+ [$C_{20}H_{18}N_4O_3S_2$], 384 (54.37) [$C_{18}H_{16}N_4O_2S_2$], 193 (100) [$C_8H_5N_2S_2$], 150 (73.69) [C_7H_6 N_2S], 134 (37.07) [C_8H_8NO], 107 (20.31) [C_6H_4O CH_3]; Anal. Calc. for $C_{20}H_{18}N_4O_3S_2$: N 13.14, S 15.04. Found: N 13.29, S 14.73

N-Benzothiazol-2-yl-N-[4-acetyl-5-(4-bromophenyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]acetamide (**7b**). Compound **6b** (3.91 g, 10 mmol) was used. Yield: 2.28 g (48%); yellow solid; m.p: 204-206°C; ^{1}H-NMR ($CDCl_3$): 2.45 (s, 3H, $COCH_3$), 2.65 (s, 3H, $COCH_3$), 7.05 (s, 1H, thiadiazoline-C_5-H), 7.27 (d, 2H, *J* = 8.4, Ar-$C_{2,6}$-H), 7.38 (t, 1H, *J* = 7.65, benzothiazole-C_6-H), 7.52-7.43 (m, 3H, benzothiazole-C_5-H and Ar-$C_{3,5}$-H), 7.58 (d, 1H, *J* = 6.85, benzothiazole-C_7-H), 7.67 (d, 1H, *J* = 7.65, benzothiazole-C_4-H); Anal. Calc. for $C_{19}H_{15}BrN_4O_2S_2 \cdot ½H_2O$: C 47.11, H 3.33. Found: C 47.13, H 2.97.

N-Benzothiazol-2-yl-N-[4-acetyl-5-(4-nitrophenyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]acetamide (**7c**). Compound **6c** (3.57 g, 10 mmol) was used. Yield: 1.46 g (33%); yellow crystals; m.p: 140-142°C; IR (cm^{-1}): 1772, 1712 (C=O), 1694 (C=N), 1602 (C=S), 1572, 1327 (NO_2); ^{1}H-NMR (DMSO-d_6): 2.22, 2.41 (two s, each 3H, two $COCH_3$), 7.38 (t, 1H, *J* = 7.65, benzothiazole-C_6-H), 7.46 (s, 1H, thiadiazoline-C_5-H), 7.50 (t, 1H, *J* =7.63, benzothiazole-C_5-H), 7.86 (d, 1H, *J* = 8.40, benzothiazole-C_7-H), 7.96 (d, 2H, *J* = 8.40, Ar-$C_{2,6}$-H), 8.05 (d, 1H, *J* = 7.65, benzothiazole-C_4-H), 8.35 (d, 2H, *J* = 8.40, Ar-$C_{3,5}$-H); Anal. Calc. for $C_{19}H_{15}N_5O_4S_2 \cdot H_2O$: N 15.24, S 13.96. Found: N 15.40, S 13.85

Schiostosomicidal assay

Schistosoma mansoni worms used for the *in vitro* screening tests were obtained after sacrifice of *S. mansoni* infected hamsters. Laboratory bred hamsters weighting 80-100 gm, maintained on a standard diet (24% protein) at the Schistosome Biological Supply Center (SBSC) at TBRI were infected percutaneously with 350 and 400 cercariae. Hamsters were usually dissected 6-7 weeks after exposure or upon the appearance of symptoms of infection maturation and the porto-mesenteric vessels were perfused using an automatic pipetting machine [19]. Worms were cleaned from blood using phosphate buffer in small 20µ mesh size sieves and rapidly placed in RPMI 1640 culture medium (2.091 L $NaHCO_3$, 0.532 g/L *N*-acetyl-*L*-glutamine, sterile filtered indotoxin tested with *L*-glutamine), to which streptomycin (300 mg), penicillin (300 units) and gentamycin (160 µg) per mL of medium were added. Sterilization was performed using an ultraviolet lamp for one hour. A weight/volume stock solution of each compound in DMSO was prepared on basis, whereby compound (10 mg) was dissolved in solvent (1 mL) to produce a 10,000 ppm solution. An aliquot of this stock solution was diluted with DMSO to prepare the test solutions at concentrations of 10, 50 and 100 µg/mL. Three vials were prepared for the concentration and five pairs of schistosome worms, males and females equally represented, were placed in each vial using sterilized forceps. In this technique [16] positive and negative controls were used. Thus, in the negative controls equal numbers of vials containing similar number of worms in a pure medium containing only DMSO were used. Praziquantel (10 ug/mL) was used for the positive controls. Test and control vials were examined for viability using a stereomicroscope. Worms that did not show motility for one minute were considered dead. The activity was measured by calculating the percentage of the number of dead worms compared to the total number of worms.

Acute toxicity

The active compounds **4a-c,** were further investigated for their oral toxicity in male mice. Five groups of mice each consisting of six animals were used for each compound. The compounds, or their

vehicle 2% gum acacia (control), were given orally in doses of 10, 50, 100 and 250 mg/kg. Twenty four hours later, the % mortality in each group was recoded.

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Sample availability: Contact the authors.