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Antiprotozoal Thiazoles. 2-(5-Nitro-2-thienyl)thiazoles

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A series of 2-(5-nitro-2-thienyl)thiazoles and their vinylogs having substituted methylamine side chains has been prepared by halogen displacement on the corresponding 4-chloromethylthiazole. Of these, 4-morpholinomethyl-2-(5-nitro-2-thienyl)thiazole showed moderate activity against *Trypanosoma cruzi* and *Trypanosoma rhodesiense* in mice. This compound formed the lead for a series of analogous thiazole-4-carboxaldehyde hydrazones. Some of the latter were found active in curing murine *Tryp. cruzi* and *Tryp. rhodesiense* infections and to have low acute toxicity. A comparison with known active compounds is given and some structural features necessary for activity are discussed.

As part of a program for the discovery of new antiprotozoal agents, we have investigated the synthesis and in vivo antitrypanosomal activity of some thiazole compounds bearing a nitro-substituted five-membered heterocyclic ring in the thiazole 2 position. In this paper, we report the synthesis of such compounds carrying a 5-nitro-2-thienyl substituent and their activity against *Trypanosoma cruzi* and *Trypanosoma rhodesiense*.

Chemistry. A search of the literature has revealed several reports of the synthesis and biological activity of 2-(5-nitro-2-furyl)thiazoles[1-3] and 2-[2-(5-nitro-2-thienyl)vinyl]thiazoles.[4] However, no description of the title compounds was found, which may be a consequence of the fact that a key intermediate, 5-nitro-2-thiophenethiocarboxamide (**1a**), was unknown. Sherman and Von Esch[3] have described the synthesis of the furan analog of **1a** in moderate yield, using the method of Taylor and Zoltewicz.[5] By a modification of this method, we obtained **1a** in 90% yield and the vinylog **1b** in 72% yield.

The treatment of compounds **1a,b** with 1,3-dichloroacetone, by a typical Hantzch[6] procedure, provided the 4-chloromethylthiazoles **2a,b** in good yield. These were formed as the free thiazoles with the evolution of gaseous HCl, the thiazole ring having a low basicity when bearing a 5-nitro-2-thienyl group.

Compounds **2a,b** were used as intermediates for the introduction of biologically active side chains. Thus, displacement of the chlorine atom in **2a,b** with a secondary amine yielded the substituted thiazolemethylamines **3a–g**. These were relatively unstable (with the exception of **3a**) and were biologically screened as their stable hydrochloride salts. See Table I.

The aldehydes **6a,b** were obtained by the treatment of **2a,b** by the method of Kröhnke[7] and, more simply, by that of Sommelet.[8] See Scheme I.

The aldehydes **6a,b** were used to broaden the scope of

Scheme I

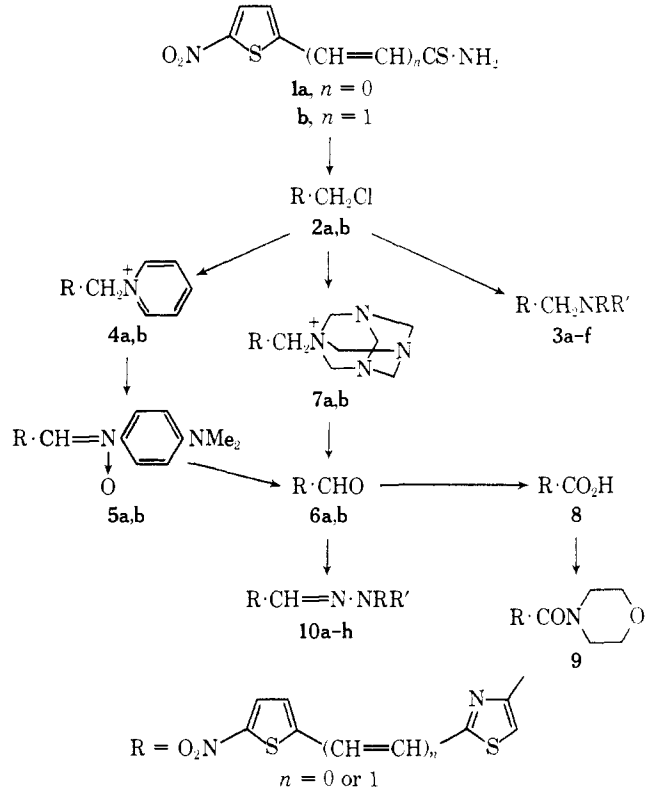
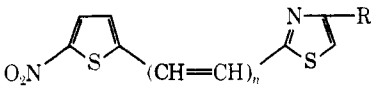


Table I. 4-Substituted 2-(5-Nitro-2-thienyl)thiazoles and Vinylogs



Compd	n	R	Recrystn solvent	Yield,[a] %	Mp, °C	Formula	Analyses
3a	0	$-CH_2$-c-$N(CH_2CH_2)_2O$	EtOH	84	130[b]	$C_{12}H_{13}N_3O_3S_2$	C, H, N
3b	1	$-CH_2$-c-$N(CH_2CH_2)_2O\cdot HCl$	Dioxane–Et_2O	56	220–223	$C_{14}H_{15}N_3O_3S_2\cdot HCl$	C, H, N
3c	0	$-CH_2$-c-$NC_5H_{10}\cdot HCl$	EtOH–Et_2O	35	215–217	$C_{13}H_{15}N_3O_2S_2\cdot HCl$	C, H, N
3d	0	$-CH_2$-c-$NC_4H_8\cdot HCl$	EtOH–Et_2O	63	219–221	$C_{12}H_{13}N_3O_2S_2\cdot HCl$	C, H, N
3e	0	$-CH_2NEt_2\cdot HCl$	EtOH–Et_2O	67	173–174	$C_{12}H_{15}N_3O_2S_2\cdot HCl$	C, N; H[c]
3f	0	$-CH_2N(CH_3)CH_2CH_2OH\cdot HCl$	Dilute HCl	41	216–218	$C_{11}H_{13}N_3O_3S_2\cdot HCl$	C, H, N
6a	0	$-CHO$	C_6H_6	74,[d] 69[e]	170–171	$C_8H_4N_2O_3S_2$	C, H, N, S
6b	1	$-CHO$	$CHCl_3$	49,[d] 47[e]	208–211	$C_{10}H_6N_2O_3S_2$	C, H, N, S
8	0	$-COOH$	DMF–MeOH	87	255–256	$C_8H_4N_2O_4S_2$	C, H, N, S
9	0	$-CO$-c-$N(CH_2CH_2)_2O$	DMF–MeOH	75	212	$C_{12}H_{11}N_3O_4S_2$	C, H, N
10a	0	$-CH{=}N$-c-$N(CH_2CH_2)_2O$	$CHCl_3$–pe[f]	80	189–190	$C_{12}H_{12}N_4O_3S_2$	C, H, N, S
10b	1	$-CH{=}N$-c-$N(CH_2CH_2)_2O$	$CHCl_3$–Et_2O	58	214–215	$C_{14}H_{14}N_4O_3S_2$	C, H, N, S
10c	0	$-CH{=}N$-c-N-$[CH_2CH(CH_3)]_2O$	$CHCl_3$–pe	67	160–164	$C_{14}H_{16}N_4O_3S_2$	C, H, N, S
10d	0	$-CH{=}N$-c-NC_5H_{10}	C_6H_6	78	194–195	$C_{13}H_{14}N_4O_2S_2$	C, H, N, S
10e	0	$-CH{=}N$-c-$N(CH_2CH_2)_2S$	$CHCl_3$–Et_2O	87	172	$C_{12}H_{12}N_4O_2S_3$	C, H, N, S
10f	0	$-CH{=}N$-c-$N(CH_2CH_2)_2SO_2$	DMF–MeOH	79	265	$C_{12}H_{12}N_4O_4S_3$	C, H, N, S
10g	0	$-CH{=}NNHCONH_2$	DMF–H_2O	84	230 dec	$C_9H_7N_5O_3S_2$	C, H, N
10h	0	$-CH{=}NNHCO$-c-$N(CH_2CH_2)_2O$	DMF–MeOH	65	117–120	$C_{13}H_{13}N_5O_4S_2$	C, H, N, S
11	0	$-COCH_3$	C_6H_6	49	198	$C_9H_6N_2O_3S_2$	C, H, N, S
12	0	$-C(CH_3){=}N$-c-$N(CH_2CH_2)_2O$	$CHCl_3$–pe	78	184	$C_{13}H_{14}N_4O_3S_2$	C, H, N, S

[a] Recrystallized material. [b] HCl salt, mp 230–232°. [c] H: calcd, 4.83; found, 5.40. [d] Via Kröhnke reaction. [e] Via Sommelet reaction. [f] pe = petroleum ether, bp 60–80°.

our side-chain modification. Thus, oxidation of **6a** with sodium dichromate in glacial AcOH gave the carboxylic acid **8**, which on treatment with $SOCl_2$ followed by morpholine gave the amide **9**.

The early finding that **3a** possessed moderate in vivo antitrypanosomal activity (Table II) prompted the use of compounds **6a,b** as intermediates for the synthesis of structural analogs of **3a**. Working on the hypothesis that a side chain with weakly basic properties conferred the antitrypanosomal activity on these thiazoles, we prepared a series of hydrazones **10a–h**, carrying similar saturated heterocyclic rings to compounds **3a–d**. Thus, condensation of **6a** with 4-aminomorpholine in $CHCl_3$ provided the highly crystalline ruby-red compound **10a**.

In order to investigate the influence of alkylation at the methine carbon atom, the ketone 11 was synthesized by the cyclization of **1a** with 1-bromobutane-2,3-dione. Subsequent condensation of 11 with 4-aminomorpholine gave the hydrazone 12 analogous to **10a**.

Biological Results and Discussion. The results of screening and acute toxicology are summarized in Table II. For details of biological screening methods, see the Experimental Section.

Compound **3a** showed a modest increase in mean survival time (MST) of 22 days against *Trypanosoma cruzi* and 12 days against *Trypanosoma rhodesiense* at a dose of 130 mg/kg ip. Comparison with the inactive compounds **3c–f** demonstrated a specific effect of the morpholine moiety in the thiazolemethylamines. It is noteworthy that other active antimicrobial nitro heterocycles have carried this group as a side chain. Thus, 5-morpholinomethyl-3-(5-nitro-2-furfurylidineamino)-2-oxazolidinone (Furaltadone)[9] and its recently described thiophene analog[10] have shown considerable antibacterial and antitrichomonal activity, respectively.

The replacement of the methylene group in **3a** by a carbonyl group, e.g., **9**, or the insertion of an ethylene bridge between the two heterocyclic rings, e.g., **3b**, abolished all antitrypanosomal activity. However, the introduction of the aldehyde hydrazone group in place of the morpholinomethyl side chain generally enhanced the activity against both species of trypanosomes and reduced the acute toxicity. The aminomorpholine derivative **10a** was the most active compound in this series, curing mice of the *Tryp. cruzi* infection at 100 mg/kg ip and 200 mg/kg po. Compound **10a** was also curative in mice against *Tryp. rhodesiense* at 200 mg/kg ip. The insertion of an ethylene bridge between the heterocyclic rings in this series also abolished activity, e.g., **10b**.

The hydrazones **10d,e** were almost equiactive with **10a** at doses of 100 mg/kg ip vs. *Tryp. cruzi* but displayed a more rapid fall of activity with dose. However, the thiomorpholine derivative **10e** was the most active compound against *Tryp. rhodesiense*. The sulfone **10f** was inactive, possibly due to its extreme insolubility. In support of this, an examination of the murine peritoneal cavity at autopsy showed that much of the material remained unabsorbed. An alkyl group on the methine carbon atom of the side chain caused great loss of activity, as shown by **12**.

Although an insufficient number of compounds of this type have been screened to evaluate a detailed structure–activity relationship, we have found a certain structural specificity necessary for antitrypanosomal activity within this series. Thus, the two heterocyclic rings should be directly linked and the thiazole ring bear a weakly basic side chain. As yet, no attempt has been made to optimize the

Table II. MST[a] of Mice Treated with 2-(5-Nitro-2-thienyl)thiazoles

	LD_{50} in mice, mg/kg		*Trypanosoma cruzi*, dose, mg/kg × 5[b]						*Trypanosoma rhodesiense*, dose, mg/kg × 4[b]					
			ip				po		ip				po	
Compd	ip	po	200	100	50	25	500	200	200	100	50	25	500	200
3a	640	1200		22[c]		0		2		12[c]		0		2
3b	260	300			0[c]						0[c]			
3c	100	300				0[c]						0[c]		
3d	100	300				0[c]						0[c]		
3e	100	300				0[c]						0[c]		
3f	300	300			0[c]						0[c]			
6a	300	600			0[c]						0[c]			
8	75	500				0[c]								
9	>800	>1600	0						0					
10a	>800	>1600	s[d]	s	27	12	s	s	s	5	2	1	10	2
10b	>800	>1600	0						0					
10c	>800	>1600	28	13	1		3	0	8	1	0		0	0
10d	>800	>1600	s	s	7	3	11	4	13	7	0	0	1	1
10e	>800	>1600	s	32	16	0	29	4	s	20	s	22	21	17
10f	>800	>1600	0						0					
10g	>800	>1600	0						0					
10h	>800	>1600	38	39	4	11	0		0	0			0	
11	>800	>1600	0						0					
12	>800	>1600	34	0			0	0	0	0			0	0
Nifurtimox[e]	>800	>1600	s	s	s	s	s	s			0			0
Pentamidine	50					0[f]						s[f]		0[f]

[a]Increase in mean survival time in days. Mean survival time of untreated mice infected with *Tryp. cruzi* = 14 (±1 day) days and with *Tryp. rhodesiense* = 4 (±0.5) days. [b]See Experimental Section. [c]Dosage in these cases = 0.2 × ip LD_{50} per day. [d]S denotes that all mice in the group survived a minimum of 60 days post-infection for *Tryp. cruzi* and 30 days for *Tryp. rhodesiense* with negative parasitemia. [e]See ref 13. [f]Dose for pentamidine = 1.25 mg/kg ip and po.

position of this side chain on the thiazole ring. This side chain is preferably of the $C(R_1)=NNR_2R_3$ type wherein R_1 must be hydrogen and R_2R_3 optimally form part of a morpholine or thiomorpholine ring. While the present compounds are clearly not as active as the standards, the oral activity and low acute toxicity are features of some potential in the treatment of trypanosomiasis.

Our work is continuing on thiazoles of this type and also others carrying alternative nitro heterocyclic rings to further define the structure having maximum antitrypanosomal activity.

Experimental Section

Melting points were determined by means of a Kofler hot-stage microscope and are uncorrected. All compounds described in this paper have ir, uv, and NMR spectra that were fully in accord with their proposed structures. Where microanalyses are indicated by the symbols of the elements only, the results observed were within ±0.4% of the theoretical values. Evaporations were performed under vacuum in a Buchi Model R rotary evaporator. All recrystallizations were carried out with the aid of decolorizing charcoal and all compounds were dried to constant weight in a vacuum oven.

5-Nitro-2-thiophenethiocarboxamide (1a). Dry HCl gas was passed rapidly into dry DMF (750 ml) with stirring, maintaining the internal temperature below 60° with a cooling bath. The gas flow was stopped when the reaction ceased to be exothermic. At this point the DMF–HCl complex crystallized completely if cooled below 40°. 5-Nitro-2-cyanothiophene[11] (92.3 g, 0.6 mol) was added followed by thioacetamide (90 g, 1.2 mol). The mixture was stirred at 40° for 3 hr, poured into H_2O (4 l.), and stirred for 30 min. The bright orange precipitate was collected, washed with H_2O by slurrying, and dried to yield 101 g (90%) of **1a**: mp 187–190°. An analytical sample, crystallized from EtOAc, had mp 189–190°. Anal. ($C_5H_4N_2O_2S_2$) C, H, N.

4-Chloromethyl-2-(5-nitro-2-thienyl)thiazole (2a). *Warning! This compound is irritant to the eyes, skin, and mucous membranes.* A mixture of **1a** (75 g, 0.4 mol) and $ClCH_2COCH_2Cl$ (101.6 g, 0.8 mol) in dry dioxane (80 ml) was stirred and heated on a steam bath under a N_2 atmosphere for 1.5 hr. During this period the mixture darkened and HCl gas was evolved. After cooling to room temperature, H_2O (1 l.) was added with stirring and the dark brown solid collected. This was washed with hot H_2O and dried giving 102 g of crude **2a**: mp 155–160°. The material was dissolved in boiling $CHCl_3$ (1 l.), charcoaled, and filtered, and the filtrate was evaporated to a volume of ca. 200 ml. The concentrated solution was cooled to 0° giving pure **2a** as yellow-orange crystals: yield 80 g (77%); mp 160°. Anal. ($C_8H_5ClN_2O_2S_2$) C, H, N.

***trans*-2-(5-Nitro-2-thienyl)thioacrylamide (1b).** The procedure was similar to that of **1a**, using *trans*-2-(5-nitro-2-thienyl)acrylonitrile (1.8 g, 0.01 mol) and thioacetamide (1.7 g, 0.02 mol) in DMF–HCl complex (20 ml). The yield was 1.55 g (72%) of red-brown crystals: mp 180°. Anal. ($C_7H_6N_2O_2S_2$) C, H, N. The acrylonitrile intermediate was obtained by the dehydration of the corresponding acrylamide with TsCl–pyridine in 50% yield: mp 115–117°.

***trans*-4-Chloromethyl-2-[2-(5-nitro-2-thienyl)vinyl]thiazole (2b)** was prepared from **1b** (1.4 g, 6.5 mmol) and $ClCH_2COCH_2Cl$ (1.6 g, 13 mmol) in dioxane (4 ml) by a similar process to that used for **2a**. The crude product was crystallized from *i*-PrOH and weighed 0.8 g: mp 142–144°. Anal. ($C_{10}H_7ClN_2O_2S_2$) C, H, N.

4-Morpholinomethyl-2-(5-nitro-2-thienyl)thiazole (3a). Compound **2a** (5.2 g, 0.02 mol) was dissolved in morpholine (10 ml) with stirring. The temperature rose spontaneously to 50° over 10 min and the solution turned dark green. The solution was diluted with H_2O (100 ml) and stirred for 10 min and the yellow-green solid collected. Crystallization from EtOH gave 5.3 g (84%) of **3a**: mp 130°. Anal. ($C_{12}H_{13}N_3O_3S_2$) C, H, N. The HCl salt of **3a** had mp 230–232°.

Compounds **3b–f** were prepared in a similar manner.

2-(5-Nitro-2-thienyl)-4-thiazolemethylpyridinium Chloride (4a). Compound **2a** (37.0 g, 0.14 mol) was suspended in dry pyridine (125 ml) and heated on a steam bath for 1 hr. The solid dissolved rapidly and was replaced by a copious yellow precipitate. The suspension was cooled and diluted with Et_2O (200 ml) and the precipitate collected: yield 46 g (95%) of **4a**; mp 254° dec. An analytical sample recrystallized from EtOH had mp 255° dec. Anal. ($C_{13}H_{10}ClN_3O_2S_2$) C, H, N, Cl, S.

***trans*-2-[2-(5-Nitro-2-thienyl)vinyl]-4-thiazolemethylpyridinium chloride (4b)** was prepared in an analogous manner to **4a** from **2b** (4.0 g, 0.014 mol). The yield of **4b** was 5.0 g (97%), mp 245–250° dec.

2-(5-Nitro-2-thienyl)-4-thiazolecarboxaldehyde (6a). Kröhnke[7] Method. NaOH (2 *N*, 125 ml) was added dropwise to a stirred suspension of **4a** (41.1 g, 0.12 mol) and *p*-$NOC_6H_4NMe_2$·HCl (22.6 g, 0.12 mol) in MeOH (200 ml) at room temperature. The color changed from yellow to green to brown, at which point a thick precipitate formed. Stirring was continued for 1.5 hr and the precipitate filtered off. After washing with H_2O and drying, the nitrone **5a** weighed 42 g and melted at 206–207°. An analytical sample was crystallized from dioxane: mp 207–208°. Anal. ($C_{16}H_{14}N_4O_3S_2$) C, H, N, S. The nitrone (39.0 g, 0.1 mol) was suspended in H_2O (400 ml), concentrated HCl (100 ml) added, and the mixture warmed to 60° for 15 min. The olive-green precipitate was collected and washed successively with dilute HCl and H_2O. The dried solid was extracted with boiling C_6H_6 (5 × 200 ml); the extracts were charcoaled, filtered, and evaporated to ca. 200 ml volume. On cooling to 20° the orange crystals were collected and dried, providing 18.5 g (74%) of **6a**: mp 170–171°. Anal. ($C_8H_4N_2O_3S_2$) C, H, N, S.

Sommelet[8] Method. Compound **2a** (39.0 g, 0.15 mol) and hexamine (40.0 g, 0.28 mol) in $CHCl_3$ (300 ml) were stirred and boiled under reflux for 20 hr. The precipitate of the hexaminium salt **7a** was filtered off, washed with $CHCl_3$, and, after drying, weighed 57.6 g (96%): mp 210–215° dec. Compound **7a** was dissolved in 50% aqueous AcOH (300 ml), boiled for 1 hr, and diluted with H_2O (1 l.) containing concentrated HCl (50 ml). The precipitate was collected, washed with H_2O, and dried. The product, weighing 24.0 g (69%), melted at 170° and was identical in all respects with that obtained from the Kröhnke reaction.

***trans*-2-[2-(5-Nitro-2-thienyl)vinyl]-4-thiazolecarboxaldehyde (6b)** was prepared by the Kröhnke method in 49% yield and by the Sommelet method in 47% yield, using processes analogous to those used for **6a**: mp 208–211°. Anal. ($C_{10}H_6N_2O_3S_2$) C, H, N, S.

2-(5-Nitro-2-thienyl)-4-thiazolecarboxylic Acid (8). Compound **6a** (7.2 g, 0.03 mol) was suspended in 50% aqueous AcOH (150 ml) containing concentrated H_2SO_4 (15 ml). A solution of $Na_2Cr_2O_7·2H_2O$ (9.0 g, 0.03 mol) in H_2O (30 ml) was added slowly and the mixture stirred and heated at 55–60° for 2.5 hr. The mixture was diluted with H_2O (600 ml) and the yellow product collected, washed thoroughly with H_2O, and dried: yield 6.7 g (87%); mp 252°. A sample crystallized from DMF–MeOH had mp 255–256°. Anal. ($C_8H_4N_2O_4S_2$) C, H, N, S.

2-(5-Nitro-2-thienyl)-4-morpholinocarbonylthiazole (9). Compound **8** (6.7 g, 0.025 mol) was boiled under reflux with thionyl chloride (15 ml) in dioxane (100 ml) until gases ceased to be evolved (2.5 hr). The dark solution was charcoaled, filtered, and evaporated to dryness. The yellow solid residue was washed with petroleum ether (bp 60–80°) and dried, yielding 5.5 g of crude acid chloride.

The acid chloride (6.7 g, 0.024 mol) in dioxane (50 ml) was added dropwise to a solution of morpholine (4.5 g, 0.05 mol) in dioxane (25 ml) at 20°. The mixture was stirred overnight and diluted with H_2O (400 ml), yielding 7.3 g of crude yellow material. Crystallization from DMF–MeOH gave 6.0 g (75%) of **9**: mp 212°. Anal. ($C_{12}H_{11}N_3O_4S_2$) C, H, N.

4-Thiazolecarboxaldehyde Hydrazones. The general method for the synthesis of the hydrazones **10a–h** is illustrated by the details for compound **10a**. Compound **6a** (4.8 g, 0.02 mol) and 4-aminomorpholine (2.5 g, 0.024 mol) were boiled together in $CHCl_3$ (50 ml) containing a few drops of glacial AcOH for 30 min. The hot solution was diluted with petroleum ether (bp 60–80°, 20 ml) and cooled to 0°. The red crystals weighed 5.5 g and were recrystallized from $CHCl_3$–petroleum ether (bp 60–80°), yielding 5.2 g of pure **10a**: mp 189–190°. Anal. ($C_{12}H_{12}N_4O_3S_2$) C, H, N, S.

The hydrazines used for the synthesis of compounds **10a–h** were either purchased or prepared by published methods.

2-(5-Nitro-2-thienyl)-4-acetylthiazole (11). A solution of **1a** (18.8 g, 0.1 mol) and 1-bromobutane-2,3-dione[12] (20.0 g, 0.12 mol) in dioxane (100 ml) was warmed to 50° for 10 min. The dark solution was diluted with H_2O (100 ml); the precipitate thus obtained was dried and extracted with hot C_6H_6 (2 × 300 ml). The extracts were charcoaled, filtered, and evaporated until crystallization began. Cooling gave 12.5 g (49%) of **11**: mp 198°. Anal. ($C_9H_6N_2O_3S_2$) C, H, N, S.

Condensation of **11** (5.1 g, 0.02 mol) with 4-aminomorpholine by a similar method to that of **10a** gave 5.3 g (78%) of hydrazone **12**: mp 184°. Anal. ($C_{13}H_{14}N_4O_3S_2$) C, H, N, S.

Antitrypanosomal Screening. The target compounds were screened for antitrypanosomal activity by the following method.

Groups of four CFW mice were infected with 2.5 × 10^5 organisms by ip injection for *Tryp. rhodesiense* and sc injection for *Tryp. cruzi*. Four hours later, the compound, in a finely divided suspension in water containing a small amount of wetting agent (Tween 80), was administered ip to one group of mice. For the *Tryp. cruzi* infection, compounds having an ip LD_{50} of <800 mg/kg were given at an ip dosage rate of ⅕ of the ip LD_{50} daily for 5 days. Compounds having an ip LD_{50} of >800 mg/kg were given at a dose rate of 200 mg/kg ip daily for 5 days.

In the case of *Tryp. rhodesiense* a similar dose regimen was given for 4 days only, owing to the rapid progress of the infection with the virulent strain used.

The antitrypanosomal activity is expressed as the increase in mean survival time in days (MST) of a group of drug-treated, infected mice over that of a control group of infected, untreated animals.

Any mice surviving a minimum of 60 days post-infection for *Tryp. cruzi* or 30 days for *Tryp. rhodesiense* were examined for peripheral parasitemia.

Those animals showing a negative parasitemia at this time were presumed cured. Compounds showing such activity were retested orally and at lower ip dose to establish a dose–response relationship. The established drugs nifurtimox[13] for *Tryp. cruzi* and pentamidine for *Tryp. rhodesiense* were included in the test program to act as standards.

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