Potential Antitrypanosomal Agents. 1,N^2-Disubstituted 2-Amino-5-hydroxy-4-methylnaphtho[1,2-*d*]thiazolium Salts and Related Compounds

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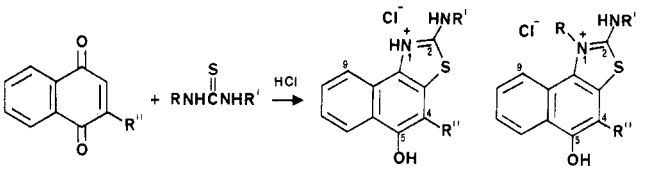
A series of 1-alkyl-2-(substituted-amino)-5-hydroxy-4-methylnaphtho[1,2-*d*]thiazoles having in vitro trypanocidal activity is described. Several caused complete lysis of *Trypanosoma brucei* organisms within 30 min at 10^{-5} M. The presence of a hydrophobic substituent on the 2-amino group was associated with high antitrypanosomal activity. Some analogues unsubstituted at the 1-position, a known class of compounds, were also active. None of the derivatives significantly prolonged the survival of *T. brucei* infected mice. Inhibition of activity in vitro by bovine serum albumin was observed. Because of the structural novelty of these agents in comparison with known trypanocides, their mechanism of action warrants further investigation.

African trypanosomiasis in livestock continues to be a serious constraint on livestock production in large areas of sub-Saharan Africa.[1] Epidemics of human trypanosomiasis in Africa earlier in this century have subsided to the present level of controlled endemicity largely due to chemotherapy, chemoprophylaxis, tsetse eradication efforts, and the avoidance of endemic areas.[2,3] Nevertheless, disease foci still exist, and there is potential for new human epidemics.[2c,d,3] Presently used trypanocides have serious drawbacks, such as toxicity or ineffectiveness in late-stage infection.[2e-h,3,4] Virtually no new trypanocides have entered clinical or veterinary use since the introduction of melarsoprol and diminazene aceturate in the 1950's.[2e,3] Because of the appearance of trypanosome strains resistant to currently used drug classes, trypanocides of new structural types are urgently needed.[1b,2e,3]

Work in this laboratory has shown that certain simple quinones have in vitro activity against bloodstream forms of *Trypanosoma brucei*.[5a] Others have reported that a number of cytotoxic anthraquinones show substantial activity against African trypanosomes in vitro.[5b] The unusual pathways of energy metabolism in these parasites appear to render them especially susceptible to the action of quinones and related agents.[6] We have prepared and tested a variety of quinoid and quinone-derived substances in order to identify trypanocidal derivatives which lack the pharmacologically undesirable electrophilic properties of quinones. In the course of these studies some N-substituted 2-amino-5-hydroxy-4-methylnaphtho[1,2-*d*]thiazoles were found to have substantial rapid trypanolytic activity in vitro. This paper describes the synthesis and antitrypanosomal screening of a series of these compounds (Table I).

Scheme I



1-6 7-26

Chemistry. Although the adducts of thioureas with quinones have been known for some time,[7] the formation of ring-fused thiazoles from these reactants was first reported by Lau and Gompf[8] in 1970. They prepared **1–3**, **5**, and other naphtho- and benzothiazoles by treating thiourea or a monosubstituted thiourea with an excess of a 1,4-naphtho- or benzoquinone in ethanolic HCl (Scheme I). In all cases described[8] (including **3** and **5**) and in **4** and **6** prepared analogously for the present study, monosubstituted thioureas afforded aminothiazole derivatives substituted on the amino group and not on the ring nitrogen. No examples using disubstituted thioureas were reported.

We found that under similar conditions a variety of N,N'-disubstituted thioureas react with 2-methyl-1,4-naphthoquinone to yield 1,N^2-disubstituted 2-amino-5-hydroxy-4-methylnaphtho[1,2-*d*]thiazolium derivatives **7–24**. The cyclic thioureas, imidazolidine-2-thione and hexahydropyrimidine-2-thione, afforded the tetracyclic naphthothiazolium compounds **25** and **26**, respectively. In the naphthothiazole products **14–24** derived from unsymmetrical N,N'-disubstituted thioureas, selective cyclization via attack by the nitrogen bearing the sterically least-demanding group was indicated by NMR data. In 1:1 D_2O–Me_2SO-d_6, the N^2,4-dimethyl derivative **3** has methyl resonances at δ 2.25 and 3.01, while the 1,N^2,4-trimethyl compound **7** has methyl resonances at δ 2.36, 3.12, and 3.95; the δ 2.25 and 2.36 resonances are assigned as 4-CH_3, the δ 3.01 and 3.12 peaks as N^2-CH_3, and the 3.95 peak of **7** as 1-CH_3. Compounds **14–22** each display two methyl singlets which appear in the ranges δ 2.0–2.36 (4-CH_3) and 3.78–4.38 (1-CH_3). Compounds **23** and **24** are assigned as

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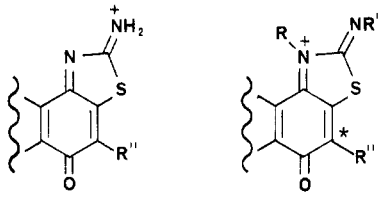
Table I. Chemical and Antitrypanosomal Properties of 2-Amino-5-hydroxynaphtho[1,2-*d*]thiazoles

no.	R	R'	R''	yield,[a] %	mp, °C	recrystn solvent[b]	formula[c]	act. against *T. brucei*: min. lytic concn,[d,e] μM	act. against *T. brucei*: max tol'd dose,[f] mg/kg
1	H	H	H	68	296–297 dec	A	*g*	>500	100
2	H	H	CH_3	67	>300	B	*h*	100	50
3	H	CH_3	CH_3	61	>300	C	*i*	30	100
4	H	$CH_2CH{=}CH_2$	CH_3	69	240–245 dec	D	$C_{15}H_{15}ClN_2OS$	20	200
5	H	C_6H_5	CH_3	23	263–264 dec	B	*j*	20	200
6	H	*p*-$C_6H_4OCH_3$	CH_3	22	235–240 dec	A	$C_{19}H_{17}ClN_2O_2S$	10	400
7	CH_3	CH_3	CH_3	56	270–280 dec	A	$C_{14}H_{15}ClN_2OS$	100	50
8	CH_2CH_3	CH_2CH_3	CH_3	50	262–263 dec	E	$C_{16}H_{19}ClN_2OS$[k,l]	50 (200)	200
9	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	CH_3	50	239–241 dec	F	$C_{18}H_{23}ClN_2OS$	30	400
10	$CH_2CH{=}CH_2$	$CH_2CH{=}CH_2$	CH_3	28	229–230 dec	E	$C_{18}H_{19}ClN_2OS$	20	400
11	$CH_2CH(CH_3)_2$	$CH_2CH(CH_3)_2$	CH_3	38	247–250 dec	G	$C_{20}H_{27}ClN_2OS$	10	200
12	$(CH_2)_3CH_3$	$(CH_2)_3CH_3$	CH_3	52	208	F	$C_{20}H_{27}ClN_2OS$	10 (100)	400
13	$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	CH_3	55	189–190	H	$C_{24}H_{35}ClN_2OS$	>10	200
14	CH_3	CH_2CH_2OH	CH_3	41	233–234 dec	A	$C_{15}H_{17}ClN_2O_2S$	100	100
15	CH_3	$CH_2CH_2OCH_3$	CH_3	44	241–242 dec	E	$C_{16}H_{19}ClN_2O_2S$	50	50
16	CH_3	$(CH_2)_3N(CH_2CH_2OH)_2{\cdot}HCl$	CH_3	31	268–270 dec	I	$C_{20}H_{29}Cl_2N_3O_3S$	100 (200)	50
17	CH_3	$(CH_2)_3$-c-$N(CH_2CH_2)_2NCH_3{\cdot}2HCl$	CH_3	50	255–258 dec	J	$C_{21}H_{31}Cl_3N_4OS$[m]	100 (100)	100
18	CH_3	$(CH_2)_3CH_3$	CH_3	43	237–238 dec	K	$C_{17}H_{21}ClN_2OS$	10	200
19	CH_3	$(CH_2)_7CH_3$	CH_3	27	224–226 dec	L	$C_{21}H_{29}ClN_2OS$	10	200
20	CH_3	$CH_2CH_2C_6H_5$	CH_3	58	229–230	E	$C_{21}H_{21}ClN_2OS$	10	400
21	CH_3	C_6H_5	CH_3	40	258–266 dec	E	$C_{19}H_{17}ClN_2OS$	10	200
22	CH_3	*m*-$C_6H_4C(=O)CH_3$	CH_3	24	210[n]	M	$C_{21}H_{18}N_2O_2S$[l,n]	ins[o]	200
23	CH_2CH_2OH	c-C_6H_{11}	CH_3	29	196–197 dec	E	$C_{20}H_{25}ClN_2O_2S$	20	400
24	CH_2CH_2OH	C_6H_5	CH_3	9	205–207 dec	E	$C_{20}H_{19}ClN_2O_2S$	>5	400
25	$-CH_2CH_2-$		CH_3	56	>300	L	$C_{14}H_{13}ClN_2OS{\cdot}H_2O$[p]	30	200
26 melarsoprol	$-CH_2CH_2CH_2-$		CH_3	27	>300	C	$C_{15}H_{15}ClN_2OS$	>100 100	200

[a] Yields are not optimized. [b] Recrystallization solvents: A = MeOH–EtOAc; B = Me_2SO–EtOAc; C = MeOH–CH_2Cl_2–Et_2O; D = MeOH–$CHCl_3$–Et_2O; E = MeOH–$CHCl_3$–EtOAc; F = EtOH–EtOAc; G = MeOH–$CHCl_3$–Me_2CO; H = EtOH–Me_2CO–Et_2O; I = water–MeOH–tetrahydrofuran; J = aqueous EtOH; K = EtOH–$CHCl_3$–EtOAc; L = *i*-PrOH–$CHCl_3$–EtOAc; M = EtOH; N = water–MeOH–Me_2CO. [c] Compounds had C, H, and N analyses within 0.4% of theory, except where noted. [d] Minimum concentration lysing all trypanosomes within 30 min in vitro. [e] Values in parentheses were determined in the presence of 0.3% added bovine serum albumin. Values preceded by a "greater than" sign denote inactivity at the solubility limit. [f] Maximum tolerated (nonfatal) ip dose tested in mice infected with *T. brucei*; no dose shown produced significant prolongation of survival, i.e., >125% of controls. [g] Literature[8] mp 294–296 °C. [h] Literature[8] mp >320 °C. [i] Literature[8] mp >321 °C dec. [j] Literature[8] mp 263–264 °C dec. [k] Also analyzed for Cl, within 0.4% of theory. [l] Also analyzed for S, within 0.4% of theory. [m] Too hygroscopic for analysis; the hydrogen oxalate salt had satisfactory analysis (see Experimental Section). [n] Purified as the free base (see Experimental Section). [o] Insoluble: inactive at 5 μM, with precipitation. [p] Anal. C: calcd, 51.14; found, 51.59.

1-(2-hydroxyethyl) derivatives, because their *N*-methylene absorptions appear at ca. δ 4.87 and 4.99, respectively, compared with ca. δ 3.65 in N^2-(2-hydroxyethyl) derivative **14**. Inspection of space-filling molecular models suggests that substituents larger than primary alkyl in the 1-position will suffer severe steric interaction with the C-9 hydrogen and the 2-amino substituent of the naphthothiazole nucleus.

In the simpler case of 1,4-naphthoquinone, only its reaction with thiourea to form **1** was reported by Lau and Gompf.[8] Although we were able to reproduce this result, we have so far been unable to isolate tractable products from the reaction of N,N′-disubstituted thioureas, such as *N,N′*-diethylthiourea or imidazolidine-2-thione, with 1,4-naphthoquinone. An explanation for this failure may be found in the proposed mechanism[8] for these reactions, which involves an oxidized intermediate of the quinone imine type, such as A in the case of cyclocondensation of



A B

thiourea itself with a quinone. In the corresponding intermediate B from the reaction of an N,N′-disubstituted thiourea with a quinone, the nitrogen of the newly formed ring would bear an obligatory positive charge. This should render the asterisked position more susceptible to Michael attack by nucleophiles, such as other reaction intermediates, chloride, or ethanol, than would be the case in A. In the adducts derived from 2-methyl-1,4-naphthoquinone, the presence of a methyl group at R″ in B would prevent addition–rearomatization reactions from competing with the proposed intermolecular reduction[8] which provides the final products, **7–26**.

Biological Results

The in vitro activity of compounds **1–26** against *T. brucei* EATRO 110 was assayed by a modification of a previously described procedure.[9] Selected concentrations in the range 5 to 1000 μM in steps of 1.5- to 2-fold dilution were tested in order to determine a minimum lytic concentration (MLC) for each agent, defined as the lowest concentration producing complete lysis of all trypanosomes within 30 min at 37 °C. Results are shown in Table I. The known trypanocide melarsoprol had an MLC of 100 μM in this system.

The parent compound 2-amino-5-hydroxynaphtho[1,2-*d*]thiazolium chloride (**1**) does not produce complete lysis even at 500 μM, its solubility limit in the test medium. The corresponding 4-methyl derivative **2** has significant activity, with an MLC of 100 μM. In the 4-methyl series bearing identical *N*-alkyl substituents at the 1-position and the 2-amino group (**7–13**), activity increases approximately with increasing side-chain size up to at least four carbons, the di-*n*-butyl and diisobutyl derivatives **12** and **11** being lytic at 10 μM. The di-*n*-hexyl analogue **13** is nonlytic at 10 μM; its assessment at higher concentrations is precluded by partial precipitation. In the case of **25** and **26**, in which N-1 and the 2-amino group are joined into a fourth ring by an ethylene or trimethylene bridge (**25** and **26**, respectively), the former is more active (30 vs. 100 μM).

In the 1,4-dimethyl series (**14–22**), larger hydrophobic 2-amino substituents, as in **18–21**, confer high activity (MLC ca. 10 μM), but aliphatic substituents containing polar heteroatoms, as in **14–17**, lead to lesser activities (MLC ca. 50–100 μM) comparable to those of the trimethyl compound **7** and the N-unsubstituted analogue **2**.

In the series of 4-methyl compounds lacking 1-substitution (**2–6**), activity increases with the size of the relatively nonpolar 2-amino substituents, the MLC's declining from 100 to 10 μM.

In three pairs of compounds, no clear pattern emerges for the effect of the presence or absence of an alkyl substituent at the 1-position (**7**, **21**, and **10** vs. **3**, **5**, and **4**). The presence of a 1-methyl group in **7** results in poorer activity vs. **3**, whereas in **21** the 1-methyl group confers greater activity compared to **5**. In the case of **10** vs. **4**, the presence or absence of a 1-allyl group had little effect on activity.

In comparing **18** and **12**, no difference in activity is seen on changing methyl to butyl at the 1-position, when a butyl group is present at the N^2-position. In contrast, for the 1-methyl derivatives **7** and **18**, a large increase in activity results on changing methyl to butyl at N^2.

Four compounds (**8**, **12**, **16**, and **17**) were also tested in the presence of 0.3% added bovine serum albumin in the suspending medium (values in parentheses in Table I). Albumin had an inhibitory effect on lytic activity; the degree of inhibition decreased with increasing polarity of the 2-alkylamino side chain.

None of the test compounds **1–26** showed significant activity against *T. brucei* infection in Swiss Webster mice at the maximum tolerated dosages (Table I).

Discussion

Of the 2-amino-5-hydroxy-4-methylnaphtho[1,2-*d*]thiazoles studied, those bearing hydrophobic substituents on the amino group showed rapid trypanolytic activity in vitro at concentrations in the 10–20 μM range (ca. 4–8 ppm), but none produced significant prolongation of the lifetime of *T. brucei* infected mice. The tenfold inhibition of the activity of dibutyl derivative **12** in vitro by approximately one-tenth (0.3%) of the physiological concentration of serum albumin suggests that binding to host proteins is a possible explanation for the inactivity of these naphthothiazoles in vivo. A similar explanation has been advanced for the behavior of miconazole, which displays in vitro but not in vivo activity against *T. brucei*, the in vitro activity being subject to inhibition by serum albumin or by whole blood.[10] Hydrophilic side-chain derivatives, such as **16** and **17**, the trypanocidal activity of which was less strongly inhibited by albumin, were, however, intrinsically less active than the nonpolar derivatives under albumin-free conditions and displayed greater host toxicity.

Because of the structural novelty of these agents in comparison with known trypanocides, their mechanism of action warrants investigation. Preliminary data indicate that naphthothiazoles of this type may inhibit trypanosomal energy metabolism. The rate of O_2 consumption by trypanosomes in vitro, measured with an O_2 electrode in a closed cell, is inhibited by 75–80% on addition of 30 μM **8** or 5 μM **12**. These concentrations, though they are immediately below the respective MLC's, do not cause substantial lysis or loss of motility. Lower concentrations cause less inhibition. Further research into the nature of this inhibition may provide leads for the design of intrin-

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sically more active analogues of these compounds.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton NMR spectra were recorded on a Varian T60A spectrometer. TLC analysis was performed on EM Laboratories silica gel 60 F-254 precoated plates. Quinones and thioureas were obtained commercially, except for those thioureas described below.

General Procedure for Unsymmetrical 1,3-Disubstituted Thioureas. A 1–2 M solution of the alkyl isothiocyanate (methyl or cyclohexyl) in ether was treated with 1 equiv of an amine. If the amine was not soluble in ether, *i*-PrOH was used as solvent. After 2–16 h, the crystalline thiourea was filtered out and washed with ether or *i*-PrOH. If the thiourea did not crystallize from the reaction mixture, the solvent was removed in vacuo and the residue was cooled and triturated with ether to induce crystallization.

***N*-(2-Hydroxyethyl)-*N'*-methylthiourea**: yield 44%; mp 74–76 °C (*i*-PrOH), lit.[11] mp 73 °C.

***N*-(2-Methoxyethyl)-*N'*-methylthiourea**: yield 81%; mp 47–48 °C (Et_2O). Anal. ($C_5H_{12}N_2OS$) C, H, N.

***N*-[3-[Bis(2-hydroxyethyl)amino]propyl]-*N'*-methylthiourea**: yield 62%; mp 88–90 °C (*i*-PrOH). Anal. ($C_9H_{21}N_3O_2S$) C, H, N.

***N*-[3-(4-Methyl-1-piperazinyl)propyl]-*N'*-methylthiourea**: yield 52%; mp 80–81 °C (*i*-PrOH–Et_2O); hydrogen oxalate, mp 217 °C (*i*-PrOH–H_2O). Anal. ($C_{10}H_{22}N_4S\cdot 2C_2H_2O_4\cdot 0.25H_2O$) C, H, N.

***N*-Methyl-*N'*-(2-phenylethyl)thiourea**: yield 75%; mp 66–67 °C (*i*-PrOH–Et_2O), lit.[12] mp 61–63 °C.

***N*-(3-Acetylphenyl)-*N'*-methylthiourea**: yield 75%; mp 119–120 °C, lit.[13] mp 120–122 °C.

***N*-Cyclohexyl-*N'*-(2-hydroxyethyl)thiourea**: yield 73%; mp 120–121 °C (*i*-PrOH). Anal. ($C_9H_{18}N_2OS$) C, H, N.

***N*-Butyl-*N'*-methylthiourea**: yield 79%; mp 41–42 °C (Et_2O–C_5H_{12}), lit.[14] mp 40–41 °C.

***N*-Methyl-*N'*-octylthiourea**: yield 73%; mp 66–67 °C (Et_2O), lit.[15] mp 58–61 °C. Anal. ($C_{10}H_{22}N_2S$), C, H, N.

General Procedure for N-Substituted 2-Amino-5-hydroxy-4-methylnaphtho[1,2-*d*]thiazoles 2–26. The method was similar to those reported[8] for the preparation of **1–3** and **5**, differing primarily in the use of more concentrated conditions. The synthesis of **8** is illustrative.

1-Ethyl-2-(ethylamino)-5-hydroxy-4-methylnaphtho[1,2-*d*]thiazolium Chloride (8). A solution of 2-methyl-1,4-naphthoquinone (13.76 g, 80 mmol) in hot absolute EtOH (100 mL) was poured into a stirred solution of *N,N'*-diethylthiourea (5.3 g, 40 mmol) and aqueous concentrated HCl (3.4 mL, 40 mmol) in absolute EtOH (50 mL). The solution was allowed to cool and was stirred at ambient temperature for 48 h. The tan precipitate that separated was filtered out and was largely freed of coprecipitated excess quinone and other impurities by successive washes with EtOH, *i*-PrOH, EtOAc, and Et_2O, affording 6.7 g (52%) of off-white powder. This was recrystallized by dissolution in hot 5:3 MeOH–$CHCl_3$ (40 mL) and dilution with hot EtOAc (400 mL). After the solution was cooled, the colorless crystalline solid was filtered off and dried, yielding 6.5 g (50%) of **8**: mp 262–263 °C dec; NMR (D_2O) δ 1.22 (t, 3), 1.42 (t, 3), 1.96 (s, 3), 3.40 (q, 2), 3.73 (br q, 2), 7.3–8.0 (m, 4); TLC R_f 0.24 (3:2 $CHCl_3$–acetone). Anal. ($C_{16}H_{19}ClN_2OS$) C, H. Cl, N, S.

Compound **22** was characterized in the form of its free base, 2-[(3-acetylphenyl)imino]-1,2-dihydro-5-hydroxy-1,4-dimethylnaphtho[1,2-*d*]thiazole, which precipitated with loss of HCl on dilution of a methanolic solution of the crude hydrochloride with water. In the synthesis of **16** and **17**, additional HCl was added to neutralize the side-chain nitrogens. The chloride form of **17**, which was used for biological tests, was too hygroscopic for analysis; it was converted by treatment with $NH_4HC_2O_4$ in aqueous MeOH to the hydrogen oxalate salt: mp 206 °C (aqueous EtOH). Anal. ($C_{21}H_{28}N_4OS\cdot 3H_2C_2O_4\cdot H_2O$) C, H, N. Further data on **1–26** are given in Table I.

Biological Methods. A. Trypanolytic Activity in Vitro. A modification of a reported procedure was used. *T. brucei* EATRO 110 bloodstream forms were isolated from blood of infected rats or mice as described previously.[16,17] Suspensions of $1.5–2 \times 10^7$ trypanosomes/mL in a 1:1 mixture of PO_4-buffered saline containing glucose (PSG 6:4 of ref 17) and minimum essential medium (GIBCO Labs) were kept on ice prior to use and were used within 8 h of isolation. Aliquots in open 1-mL cuvettes maintained at 37 °C were monitored turbidometrically at 750 nm on a Beckman Acta CIII spectrophotometer. When turbidity was stable (5–10 min), the test compound in Me_2SO, aqueous Me_2SO, or H_2O was added with mixing to achieve the desired final concentration selected from the range 1000, 500, 300, 200, 100, 50, 30, 20, 10, 5 μM. In no case did the final concentration of Me_2SO exceed 1% v/v, a level not affecting turbidity or trypanosome motility. Small aliquots removed at 5, 10, and 30 min after drug addition were examined by phase microscopy for lysis as indicated by loss of motility and loss of phase lucency. Precipitated crystals of test compound, it present, were also noted. Lysis was well correlated with loss of turbidity. The lowest concentration producing complete lysis within 30 min was designated the minimum lytic concentration (MLC). Concentrations near the MLC were replicated using two to three different passage harvests of trypanosomes.

B. Antitrypanosomal Testing in Vivo. Compounds were screened in *T. brucei* infected Swiss-Webster female mice weighing 20–25 g according to a reported procedure.[18] Briefly, mice were infected ip with 5×10^4 *T. brucei* EATRO 110 organisms using freshly drawn infected mouse blood diluted with minimum essential medium. To groups of five mice, test compounds as milled suspensions in normal saline containing 0.25% methylcellulose or as solutions in normal saline were administered ip 4–6 h after infection, at which time the presence of infective trypanosomes in the host bloodstream is demonstrable by subinoculation. Untreated controls (5–10 mice per run) died 3.9 ± 0.5 days after infection. Test dosages producing mean survival times >125% of controls are retested. Dosages causing mean survival times <90% of controls or at which deaths occurred within 72 h of infection were considered toxic, and the test compound was retested at a lower dose. Dosages were selected from the range 400, 200, 100, 50, 25 mg/kg, after a preliminary toxicity screen to estimate a maximum tolerated dose. Wet films of tail blood were inspected for trypanosomes on day 3 after infection.

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