87419-67-8; (*R*)-(−)-**1d** hydrogen di-*p*-toluoyl-*d*-tartrate, 87419-68-9; (*S*)-(+)-**1d**, 87419-69-0; (*S*)-(+)-**1d** hydrogen di-*p*-toluoyl-*l*-tartrate, 87419-70-3; (±)-1 (X = N_3; R = OMe), 87351-87-9; (±)-**3**·HCl, 64124-23-8; (±)-4, 87351-88-0; (±)-**5**, 87351-89-1; (±)-**6**, 87351-90-4; (±)-**6**·HCl, 87351-91-5; (±)-7, 87351-92-6; (±)-**8**, 87371-37-7; (±)-**9**, 87351-93-7; (±)-**9** hydrogen fumarate, 87351-94-8; (±)-**10**, 87351-95-9; (±)-11, 87419-71-4; (±)-11 (*N*-methyl derivative), 87419-72-5; (±)-**12**, 87351-96-0; (±)-**12**·HCl, 87351-97-1; (±)-**13**, 87351-98-2; (±)-14·HCl, 87351-99-3; 2-nitrobenzaldehyde, 552-89-6; 2-bromobenzaldehyde, 6630-33-7.

Supplementary Material Available: Description of experimental procedures (data collection, data reduction, and structure solution and refinement), tables of experimental details (crystal data, intensity measurements, and structure solution and refinement), positional and thermal parameters, general temperature factor expressions (*B*'s), bond distances, bond angles, torsional angles, intermolecular contacts up to 3.50 Å, least-square planes, intensity data, and a figure of a single molecule showing 40% probability ellipsoids are available (23 pages). Ordering information is given on any current masthead page.

Trypanocidal 1,3-Arylene Diketone Bis(guanylhydrazone)s. Structure–Activity Relationships among Substituted and Heterocyclic Analogues

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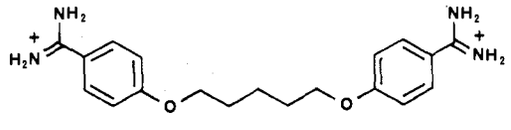
Based on the antitrypanosomal activity of 1,3-diacetylbenzene bis(guanylhydrazone) (**4**) and 2,6-diacetylpyridine bis(guanylhydrazone) (**17**), a number of substituted and heterocyclic 1,3-arylene diketone bis(guanylhydrazone)s were prepared and tested against *Trypanosoma brucei* infections in mice. A wide range of ED_{50} values was observed among 5-substituted derivatives of **4**. The 5-amino analogue **5** and 5-acetamido analogue **6** were about twice as active as **4**. 1,3,5-Triacetylbenzene tris(guanylhydrazone) (**12**) was about 9 times as active as **4** and was approximately one-half as active as the currently used trypanocide diminazene aceturate in this test system. Other 5-derivatives had activity equivalent to or less than that of the parent compound **4**. Three new heterocyclic analogues were all less active than 2,6-diacetylpyridine derivative **17** and benzene derivative **4**. Ring substitution ortho to the guanylhydrazone side chains was invariably detrimental to activity. Side-chain homologues 1,3-dipentanoylbenzene bis(guanylhydrazone) and 1,3-diacetylbenzene bis(2-imidazolin-2-ylhydrazone) were essentially inactive.

Trypanosomiasis kills over 3 000 000 head of cattle in Africa every year;[1] in addition, there are thought to be at least 10 000 new cases annually of human trypanosomiasis in Africa, although quantitation is difficult because new outbreaks tend to occur in areas of political and economic turmoil.[2] Over the last 25 years, research on the chemotherapy of African trypanosomiasis has been sufficiently quiescent that no new trypanocides have been brought into use.[3]

Bis(benzamidine) derivatives, such as pentamidine (**1**)



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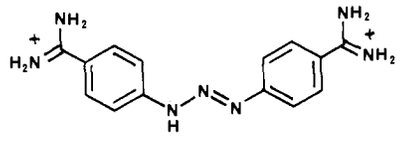

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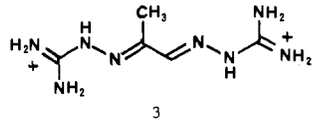

3

isethionate and diminazene (**2**) aceturate, are an important class of currently employed antitrypanosomal agents.[4] Methylglyoxal bis(guanylhydrazone) (MGG, **3**) dihydrochloride, an antitumor agent that formally resembles **1** and **2** in having two terminal amidine moieties, has been shown to possess modest trypanosuppressive properties.[5] It occurred to us that simple aromatic analogues of **3** might be better trypanocides, based on the aromatic character of **1** and **2** and of other classes of cationic trypanocides, such as quinapyramine. This conjecture proved to be correct. In our preliminary study of aromatic bis(guanylhydrazone)s,[6] 1,3-diacetylbenzene bis(guanylhydrazone) (**4**) and 2,6-diacetylpyridine bis(guanylhydrazone) (**17**) showed substantial curative activity against *Trypanosoma brucei* infection in mice; **4** was also curative against *T. congolense*. Analogous dialdehyde derivatives and 1,4-isomers were less active.[6] The 1,3-arylene diketone bis(guanylhydrazone) series was therefore selected for further study. In an effort to identify structural modifications that would enhance antitrypanosomal activity in this series, we have synthesized and tested 12 ring-substituted analogues and two side-chain homologues of **4**, as well as three new heterocyclic derivatives.

Chemistry. Guanylhydrazones **4**–**22** (Table I) were routinely prepared from the corresponding diketone and a 10–20% excess of the appropriate aminoguanidine salt in aqueous ethanol containing a trace of excess acid.

A number of 1,3-phenylene diketones required for this study apparently had not been described previously (Table II). 5-Nitro-1,3-diacetylbenzene (**29**) was prepared from 5-nitroisophthaloyl dichloride by condensation with diethyl magnesiomalonate and subsequent hydrolytic decarboxylation, by analogy with a procedure for the synthesis of 2-nitro-1,3-diacetylbenzene.[7] 4-Nitro-1,3-diacetylbenzene (**30**) and 5-methyl-1,3-diacetylbenzene (**27**) were similarly prepared from their corresponding acid dichlorides.

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(4) Williamson, J. In "The African Trypanosomiases"; Mulligan, H. W., Ed.; Wiley-Interscience: New York, 1970; pp 125–221.
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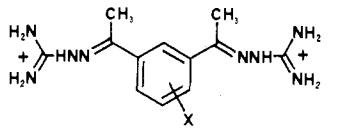
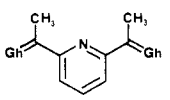
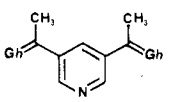
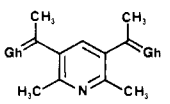
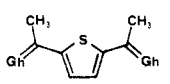
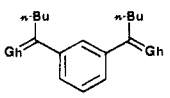
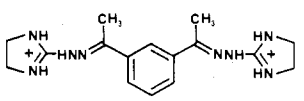
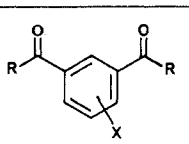
Table I. Properties of 1,3-Arylene Diketone Bis(guanylhydrazone)s



no.	X	yield, %	mp, °C	recrystn solvent[a]	formula[b]
4	H	83	330-333 dec	E-W	$C_{12}H_{18}N_8 \cdot 2HCl \cdot H_2O$
5	5-NH_2	74	355-360 dec	E-W	$C_{12}H_{19}N_9 \cdot 2HCl$
6	5-$NHCOCH_3$	75	322-325	E-W	$C_{14}H_{21}N_9O \cdot 2HCl \cdot 2H_2O$
7	5-OH	62	330-337 dec	E-W	$C_{12}H_{18}N_8O \cdot 2HCl \cdot 2H_2O$
8	5-OCH_3	80	314-316 dec	E-W	$C_{13}H_{20}N_8O \cdot 2HCl \cdot 0.5CH_3CH_2OH$[c]
9	5-CH_3	88	345-350 dec	E-W	$C_{13}H_{20}N_8 \cdot 2HCl \cdot H_2O$
10	5-I	70	235-237	E-W	$C_{12}H_{17}N_8I \cdot 2HCl \cdot 0.5H_2O$
11	5-NO_2	92	355-363 dec	E-W	$C_{12}H_{17}N_9O_2 \cdot 2HCl \cdot H_2O$
12	5-CCH_3=NNHC(=NH)NH_2·HCl	90	345-352 dec	M-W	$C_{15}H_{24}N_{12} \cdot 3HCl \cdot H_2O$
13	2-NH_2	77	335-338 dec	E-W	$C_{12}H_{19}N_9 \cdot 2HCl$
14	2-NO_2	48	292-295 dec	E-W	$C_{12}H_{17}N_9O_2 \cdot 2HCl$
15	4-NO_2	79	258-263 dec	E-W	$C_{12}H_{17}N_9O_2 \cdot 2HCl \cdot 0.5H_2O$
16	4,6-$(OH)_2$	85	335-337 dec	E-W	$C_{12}H_{18}N_8O_2 \cdot 2HCl \cdot H_2O$
	structure[d]				
17		94	360-370 dec	E-W	$C_{11}H_{17}N_9 \cdot 2HCl \cdot H_2O$
18		85	370-372 dec	E-W	$C_{11}H_{17}N_9 \cdot 2HCl$
19		50	188-190 dec	E-W	$C_{13}H_{21}N_9 \cdot 2CH_3SO_3H \cdot H_2O$
20		42	340-350 dec	I-W	$C_{10}H_{16}N_8S \cdot 2HCl \cdot H_2O \cdot 0.5(CH_3)_2CHOH$[c]
21		73	293-295 dec	E-W	$C_{18}H_{30}N_8 \cdot 2HCl$
22		58	368-370 dec	E-W	$C_{16}H_{22}N_8 \cdot 2HBr \cdot 1.5H_2O$

[a] E = ethanol; M = methanol; I = 2-propanol; W = water. [b] All compounds analyzed for C, H, and N within 0.4% of theoretical values. [c] Solvent inclusion confirmed by NMR. [d] Gh = NNHC(=NH)NH_2·HX.

Table II. 1,3-Arylene Diketones



no.	X	R	mp, °C	recrystn solvent	formula	anal.[a]
23	5-NH_2	CH_3	143-144	2-PrOH	$C_{10}H_{11}NO_2$	C, H, N
24	5-$NHCOCH_3$	CH_3	179-180	[b]	$C_{12}H_{13}NO_3$	C, H, N
25	5-OH	CH_3	150-151	EtOAc-C_6H_6	$C_{10}H_{10}O_3$	C, H
26	5-OCH_3	CH_3	96-97	subl[c]	$C_{11}H_{12}O_3$	C, H
27	5-CH_3	CH_3	36-37	subl[c]	$C_{11}H_{12}O_2$	C, H
28	5-I	CH_3	128-129	2-PrOH-EtOAc	$C_{10}H_9IO_2$	C, H
29	5-NO_2	CH_3	99-100	EtOH	$C_{10}H_9NO_4$	C, H, N
30	4-NO_2	CH_3	69-69.5	2-PrOH	$C_{10}H_9NO_4$	C, H, N
31	H	$(CH_2)_3CH_3$	27-29	subl[c]	$C_{16}H_{22}O_2$	C, H

[a] Elemental analyses were within ±0.4% of theoretical values. [b] Washed with CH_2Cl_2. [c] Purified by sublimation under reduced pressure.

Reduction of **29** with tin(II) chloride in hydrochloric acid afforded 5-amino-1,3-diacetylbenzene (**23**) by analogy with the reported procedure for the 2-amino isomer.[7] Amino dione **23** served as a common intermediate for preparing several other 5-substituted 1,3-diacetylbenzenes via standard methodology. Diazotization of **23**, followed by hydrolysis or iodination of the diazonium intermediate, gave, respectively, the phenol **25** and the iodo dione **28**. Acetylation of **23** gave acetamide derivative **24**. Methylation of phenol **25** with excess diazomethane gave methoxy

dione **26**. 1,3-Dipentanoylbenzene (**31**) was prepared by reaction of isophthalaldehyde with butyllithium, followed by oxidation of the resulting diol with manganese dioxide.

Results

The relative trypanocidal activities of bis(guanylhydrazone)s **4–22** were assessed by administration to *T. brucei* infected Swiss–Webster mice in a modification[8] of our preliminary test system.[6] The results are presented in Table III. Activities varied widely among the 12 ring-substituted analogues (**5–16**) of 1,3-diacetylbenzene bis(guanylhydrazone) **4**. On the basis of ED_{50} values, the five analogues **5–9** (5-NH_2, 5-$NHCOCH_3$, 5-OH, 5-OCH_3, and 5-CH_3, respectively) were relatively close to **4** in activity, with the nitrogenous 5-derivatives **5** and **6** being somewhat more active. Two compounds with hydrophobic electron-withdrawing groups, the 5-iodo derivative **10** and the 5-nitro analogue **11**, showed curative activity only at toxic levels and were clearly less active than the unsubstituted **4**. The presence of a third guanylhydrazonoethyl group in the trisymmetric analogue 1,3,5-triacetylbenzene tris(guanylhydrazone) (**12**) resulted in approximately a 9-fold increase in activity relative to **4**. Under these conditions, **12** was about one-half as active as the currently used veterinary trypanocide **2**.

In compounds **13–16**, with substitution ortho to the guanylhydrazone side chains [2-NH_2, 2-NO_2, 4-NO_2, and 4,6-$(OH)_2$, respectively], only the 4-nitro derivative **15** had curative activity, about one-fifth that of the parent **4**.

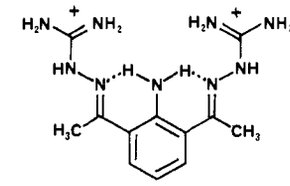
Four heterocyclic congeners of **4** were studied. Of these, only 2,6-diacetylpyridine bis(guanylhydrazone) **17** had equivalent activity. The 3,5-diacetylpyridine analogue **18** and the 2,5-diacetylthiophene derivative **20** were considerably less active than **4** or **17**. In compound **19**, the 2,6-dimethyl derivative of **18**, curative activity was lost completely.

An analogue of **4** having longer alkyl side chains, 1,3-dipentanoylbenzene bis(guanylhydrazone) (**21**), was devoid of curative activity. Also inactive was **22**, which has 2-imidazolin-2-yl moieties in place of the terminal amidines of **4**.

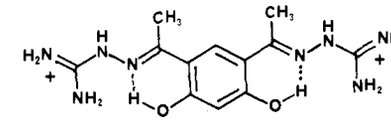
Discussion

While several 5-substituted analogues of the parent 1,3-phenylene derivative **4** had equivalent (**8** and **9**) or greater (**5**, **6**, and **12**) trypanocidal activity, substitution ortho to the guanylhydrazone side chains of **4** was uniformly detrimental (**13–16**; cf. also **18** vs. the dimethyl derivative **19**). Interestingly, although a complete lack of curative activity was observed for compounds having substitution ortho to both side chains (**13**, **14**, **16**, and **19**), some curative effects were seen in 4-nitro derivative **15**, in which the substituent is ortho to only one of the guanylhydrazone moieties.

The detrimental effect of ortho substitution occurred both with H-bonding donor substituents (NH_2 in **13**, OH in **16**) and non-H-donor groups (NO_2 in **14** and **15** and CH_3 in **19**). Intramolecular hydrogen bonding in **13** and **16** might be expected to result in dissimilar preferred conformations **13a** and **16a**; in both of these, the hydrazone C=N bonds and the aromatic ring are coplanar. In contrast, ortho nonbonding interactions in **14**, **15**, and **19** may be expected to force the adjacent hydrazone side chains out of coplanarity with the ring. Both types of ortho interaction would restrict free rotation of the guanylhydrazone side chain. The fact that all ortho interactions studied correlate with loss of activity suggests a requirement of such rotational freedom for optimal antitrypanosomal properties. Energy-dependent uptake of pentamidine (**1**) is known to occur in trypanosomes.[9] Conformational flexibility would be an advantage for analogues of **4** if the conformation preferred for an uptake system were different from the conformation preferred in an active site. An alternative possibility is that the ortho ring positions must be accessible for close van der Waals contact in a drug binding site.



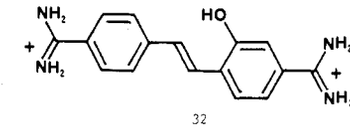
13a



16a

Present evidence does not permit an analogy to be drawn between the antitrypanosomal activity of the title compounds and the cytotoxic effects of methylglyoxal bis(guanylhydrazone) (**3**); indeed, there are dissimilarities between the antitrypanosomal and antineoplastic properties of **3** itself. In particular, the synergism observed between **3** and α-(difluoromethyl)ornithine in several neoplastic systems[10–12] is reportedly absent in a *T. brucei* system.[13]

Although the trypanocidal mechanisms of bis(benzamidine)s, such as **1** and **2**, remain unclear, nonintercalative interactions with DNA are thought to play an important role.[14,15] Diminazene (**2**) interferes with the replication of extranuclear (kinetoplast) DNA in African trypanosomes[15] and inhibits RNA synthesis in *T. brucei*.[16] On the basis of spectroscopic evidence, it has been proposed that hydroxystilbamidine (**32**), another bis(benz-



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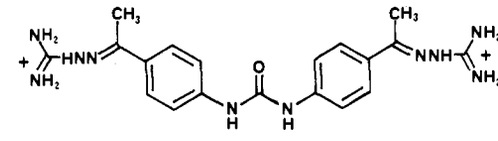

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Table III. Trypanocidal Activity of 1,3-Arylene Diketone Bis(guanylhydrazone)s in Mice

no.	ED_{50} (SD),[b] mg/kg[b]	cures/total mice [toxic deaths][a] at the following dosages												
		dosages of 150–1.5 mg/kg											lower dosages	
		150	100	60	40	25	15	10	6	4	2.5	1.5	mg/kg	c/tot[c]
4	4.2 (3.5-5.1)		1/5 [4]	7/10 [3]	9/10 [1]	10/10	10/10	22/30	32/45	24/45	6/25	0/10		
5	2.8 (2.4-3.3)			0/5 [5]	1/5 [4]	10/10	10/10	19/20	26/30	20/30	21/35	2/25	1.0	0/10
6	2.8 (2.4-3.2)				0/5 [5]	8/10 [1]	9/10	10/10	9/10	22/30	15/30	3/30	1.0	0/10
7	7.3 (5.7-9.4)			0/5 [5]	1/5 [3]	7/10 [3]	16/20	14/20	9/20	0/5				
8	5.4 (4.6-6.4)		0/5 [4]	1/10 [7]	10/10	10/10	10/10	17/20	14/30	10/30	5/30	0/20		
9	4.1 (3.5-4.8)		0/5 [5]	0/5 [4]	10/10	8/10	9/10	10/10	14/20	11/20	3/20	0/15		
10			0/5 [4]	2/5 [3]	0/10									
11		1/5 [4]	3/10 [6]	9/25 [4]	12/25 [6]	1/20 [4]	0/10	0/5	0/5					
12	0.47 (0.40-0.55)			0/5 [5]	0/5 [4]	4/5 [1]	10/10	10/10	20/20	9/10	20/20	10/10	1.0	8/10
													0.6	20/30
													0.4	19/35
													0.25	0/30
													0.15	0/10
13					0/5 [5]	0/5	0/5							
14					0/5 [5]	0/5 [3]	0/10							
15	20 (17-24)			0/5 [5]	5/5	10/15	4/15	0/5						
16		0/5 [5]	0/5		0/5		0/5							
17	4.1 (3.4-4.9)		0/5 [5]	3/5 [2]	10/10	18/20 [1]	19/20	20/20	17/20	10/20	1/10	0/10		
18					1/5 [4]	4/5 [1]	11/20	1/20	0/10					
19		0/5 [4]	0/5 [1]	0/5	0/5		0/10							
20	14 (12-17)		1/5 [4]	5/5	13/15	16/20	14/25	11/30	0/20	0/10				
21				0/5 [4]	0/5 [1]	0/15								
22					0/5 [5]	0/5 [1]	0/5 [2]	0/5						
2[d]	0.24 (0.17-0.34)	3/5 [2]	5/5	5/5	5/5	3/5	3/5	5/5	10/10	10/10	9/10	19/20	1.0	9/10
													0.6	23/25
													0.4	24/45
													0.25	28/40
													0.15	6/20
													0.1	1/10
													0.06	0/10

[a] Cures plus parasitemic deaths plus toxic deaths equals total mice. See Experimental Section for details. [b] Dosage producing aparasitemic survival to 28 days in 50% of mice with 95% confidence limits; drugs given in salt forms shown in Table I. [c] Cures/total mice. [d] Diminazene aceturate (Berenil).

Table IV. Maximal Intercationic Distances for Several Dicationic Trypanocides[a]

no.	compound	d, Å
3	methylglyoxal bis(guanylhydrazone)	8.5
4	1,3-diacetylbenzene bis(guanylhydrazone)	12
2	diminazene	12.5[b]
32	hydroxystilbamidine	12.5[c]
1	pentamidine	17
33	4,4'-diacetyldiphenylurea bis(guanylhydrazone)	19.5[d]

[a] Measured between amidine carbons on CPK space filling models. [b] References 14 and 15 give 13 Å. [c] Reference 18 gives 13 Å. [d] Reference 21 gives 19.5 Å.

amidine) trypanocide, binds to the minor groove of helical DNA such that the terminal amidines form ion pairs with phosphate groups of opposite chains while the relatively hydrophobic central portion of the molecule is presented to the interior of the groove.[17,18] Since minor-groove binding has been proposed as a physiological role for polyamines,[19] the bis(benzamidine)s may thus interfere with polyamine function. In fact, exogenous polyamines can inhibit the antitrypanosomal action of various cationic trypanocides at levels which do not inhibit trypanocide uptake.[13,20] Inspection of space-filling (CPK) models suggests that minor-groove binding should be just as feasible for **4** and analogues as for the bis(benzamidine)s. Model measurements show that the intercationic distances between terminal amidine carbons in maximally extended conformations of **2**, **4**, and **32** are very similar (Table IV); it is interesting to note that the maximal intercationic distances of these three compounds are intermediate between those of **3** and another antineoplastic bis(guanylhydrazone), 4,4'-diacetyldiphenylurea bis(guanylhydrazone) (**33**).[21-23] Recently, Bacchi et al.[13] have found that **33** also has curative activity against *T. brucei* infection in mice.

Perhaps the most unexpected finding of the present study was that the presence of a third guanylhydrazone side chain in the trisymmetric analogue **12** resulted in a 9-fold enhancement of activity against *T. brucei* relative to the bis derivative **4**. To our knowledge, trypanocidal effects have not previously been reported for any compound having three terminal amidine or guanidine groups. Indeed, 1,3,5-triguanidinobenzene was reported to be inactive toward a strain of *T. equiperdum*, against which 1,4-diguanidinobenzene was curative in mice.[24] Models indicate that **12** can assume conformations that would allow all three of its cationic centers to pair with phosphates lining the minor groove of DNA. Whatever the site of action may be for **4** and its analogues against trypanosomes, the third cationic group present in **12** can clearly be accommodated. The enhanced activity of **12** in this *T. brucei* system suggests several directions for further research, which we are currently pursuing.

Experimental Section

Melting points were determined on an Electrothermal heated block apparatus and are uncorrected. Proton NMR spectra were recorded on a Varian T60A spectrometer, in dimethyl-d_6 sulfoxide (for guanylhydrazones) or $CDCl_3$. Microanalyses were performed by the Microanalytical Service of Rockefeller University or by Schwarzkopf Microanalytical Laboratories, Woodside, NY. Reported procedures were used for the preparation of 2,6-diacetylaniline,[7] 1,3-diacetyl-2-nitrobenzene,[7] 1,3-diacetyl-4,6-dihydroxybenzene,[25] 3,5-diacetylpyridine,[26] and 3,5-diacetyl-2,6-dimethylpyridine.[27,28] Other 1,3-arylene diketones were obtained commercially.

1,3-Diacetyl-5-nitrobenzene (29). Under N_2, Mg turnings (6.56 g, 0.27 mol) were stirred with EtOH (35 mL) and catalytic CCl_4 (0.5 mL) until rapid H_2 evolution began. Diethyl malonate (43.25 g, 0.27 mol) in Et_2O was added, and the mixture was stirred until the Mg had dissolved (3 h). 5-Nitroisophthaloyl dichloride (28 g, 0.113 mol) in tetrahydrofuran (80 mL) was added dropwise at a rate causing moderate reflux. After 16 h under gentle reflux, the mixture was cooled with an ice bath and cautiously acidified with 10% aqueous H_2SO_4. The organic layer was washed with brine and dried ($MgSO_4$). Concentration gave a viscous oil, which was treated with EtOH (200 mL). The intermediate tetraethyl 5-nitroisophthaloylbis(malonate) separated as needles, mp 87–89 °C (49.9 g, 84%). The NMR of this ($CDCl_3$) was consistent with the enol form of the expected diketo tetraester. Without further characterization the tetraester was dissolved in a mixture of HOAc (200 mL), water (40 mL), and H_2SO_4 (10 mL) and heated at reflux for 3.5 h, at which time CO_2 evolution had ceased. (Larger proportions of water allow dearoylation to compete with decarboxylation and should be avoided.) The ice-cooled solution was diluted with water (500 mL). The title nitro dione separated as needles (11.0 g). An additional crop (4.3 g) was obtained by extraction of the filtrate with EtOAc: total yield 65% from the acid chloride. Recrystallization from ethanol gave analytically pure material (Table II).

Analogously, the following two diones were prepared from the corresponding substituted isophthaloyl dichlorides: 1,3-diacetyl-5-methylbenzene (**27**), 58% yield; 1,3-diacetyl-4-nitrobenzene (**30**), 76% yield (Table II).

1,3-Diacetyl-5-aminobenzene (23). The nitro dione **29** (14.7 g, 71 mmol) was added as a solid to a solution of $SnCl_2 \cdot 2H_2O$ (64 g, 284 mmol) in concentrated HCl (180 mL) at 50 °C, and external heating was removed. An exotherm to 95 °C occurred within 2 min. After 5 min, ice cooling was applied. The cooled mixture was poured cautiously (**foaming**!) onto $K_2CO_3 \cdot H_2O$ (220 g) and ice–water (400 mL). The mixture was extracted with EtOAc (3 × 400 mL). The extracts were washed with 5% $NaHCO_3$ and brine, dried ($MgSO_4$), decolorized with carbon, and filtered. The filtrate was concentrated in vacuo to give 11.2 g (89%) of the bright yellow amino dione. Recrystallization from *i*-PrOH gave analytically pure material (Table II).

1,3-Diacetyl-5-(acetylamino)benzene (24). Amino dione **23** (1.035 g, 5.0 mmol) was suspended in CH_2Cl_2 (15 mL) and treated dropwise with Ac_2O (0.94 mL, 10 mmol). The mixture was heated at reflux for 30 min. The cooled suspension was filtered, and the white precipitate was washed freely with CH_2Cl_2. Drying in vacuo gave analytically pure **24** (1.06 g, 85%, Table II).

1,3-Diacetyl-5-hydroxybenzene (25). A solution of amino dione **23** (0.866 g, 5 mmol) in 3% (v/v) H_2SO_4 (15 mL) at 0 to 5 °C was treated with solid $NaNO_2$ (0.38 g, 5.5 mmol). After 30 min at 0 to 5 °C urea (50 mg, 0.9 mmol) was added to destroy remaining HNO_2. The mixture was allowed to warm to room temperature and then was heated to 80 °C over 30 min, at which point N_2 evolution appeared complete. The mixture was cooled, saturated with NaCl and extracted with EtOAc (3 × 50 mL). The

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combined extracts were washed with brine, dried ($MgSO_4$), decolorized, and filtered. Concentration yielded 0.745 g (85%) of **25** as an off-white powder, which was recrystallized from EtOAc–C_6H_6 (Table II).

1,3-Diacetyl-5-iodobenzene (28). Diazotization of **23** (708 mg, 4.0 mmol) was carried out as in the preceding experiment. The ice-cold diazonium salt solution was then added dropwise to a solution of NaI (0.75 g, 5.0 mmol) in water (10 mL) at 5 °C with stirring; a brown precipitate separated. The mixture was stirred for 5 min, then CH_2Cl_2 (25 mL) was added, and stirring was continued for 30 min. The organic layer was separated and washed with saturated aqueous Na_2SO_3 and brine. The CH_2Cl_2 solution was filtered through carbon and $MgSO_4$ and concentrated to an orange paste, which was recrystallized from *i*-PrOH–EtOAc to yield pale orange needles (395 mg, 34%) (Table II).

1,3-Dipentanoylbenzene (31). *n*-Butyllithium in hexane (2.2 m, 13.6 mL, 30 mmol) was added dropwise to a solution of isophthalaldehyde (1.34 g, 10.0 mmol) in ether (10 mL) at 0 °C. After 15 min, the turbid mixture was poured into ice–water and extracted with CH_2Cl_2. The extracts were filtered through $MgSO_4$ and concentrated to give 1.75 g of crude 1,3-bis(1-hydroxypentyl)benzene, which was not further characterized. This intermediate (1.65 g) was dissolved in CH_2Cl_2 (75 mL) containing active MnO_2 and heated at reflux for 3 days. Filtration and concentration yielded 1.30 g of an oil. Chromatography on silica gel with chloroform afforded 0.70 g of the title dione (Table II): 30% yield from isophthalaldehyde.

General Procedure for the Synthesis of Guanylhydrazones. To a hot solution of the carbonyl compound in EtOH (2 mL/mmol) was added an aqueous 30–40% solution of recrystallized aminoguanidine hydrochloride, in 10–30% excess per carbonyl group. A catalytic amount of concentrated HCl was usually added (1–5 mol %). The reaction may be monitored by TLC (polyamide, EtOH–*i*-PrOH mixtures as eluant). Upon completion (4–48 h), the reaction was cooled, and the crystalline product that separated was filtered out, washed with EtOH, and dried. If no product separated on cooling, *i*-PrOH was added to induce crystallization. The products were recrystallized from alcohol–water.

Aminoguanidine methanesulfonate was used for the synthesis of **19**. 2-Hydrazino-2-imidazoline hydrobromide was used for the synthesis of **22**.

Antitrypanosomal Testing in Vivo. The screening procedure is essentially that described previously.[8] Female Swiss–Webster mice weighing 20–25 g were infected intraperitoneally with 5×10^4 *T. brucei* EATRO 110 organisms using freshly drawn mouse blood diluted with minimum essential medium. To groups of 5 or 10 mice were administered test compounds, as solutions or milled suspension in normal saline (20 μL/g of body weight), intraperitoneally once 6 h after infection, at which time bloodstream parasitemia was demonstrable by subinoculation. Untreated controls invariably died between 72 and 144 h after infection. Deaths of treated animals within 72 h after infection are ascribed to drug toxicity. For active compounds, dosages tested ranged from lethally toxic levels to noncurative levels at an average interval of 0.2 log unit (Table III). Survivors were observed for 28 days after infection, at which time wet films of tail blood were examined by phase contrast microscopy for trypanosomes. Survivors to 28 days lacking patent bloodstream parasitemia were counted as cures. ED_{50} ranges (Table III) were calculated by a computer program based on the methods of Bliss[29] and of Litchfield and Wilcoxon;[30] dosages at or above toxic levels were not used in these calculations.

Diminazene aceturate (Berenil, Calbiochem-Behring Corp.) was used as a positive control; its solutions were prepared immediately prior to use, because this material does not contain the antipyrine stabilizer found in veterinary Berenil.[15]

Acknowledgment. We thank I. D. Fisch, M. V. Wilson, and C. Warren for technical assistance and T. Bella for microanalyses. This research was supported by grants from the Rockefeller Foundation (RF 82043) and from the UNDP/World Bank/WHO Programme for Research and Training in Tropical Diseases.

Registry No. 4, 87533-31-1; 4 (free base), 81913-30-6; **5**, 87533-32-2; **5** (free base), 87533-59-3; **6**, 87533-33-3; **6** (free base), 87533-60-6; **7**, 87533-34-4; **7** (free base), 87533-61-7; **8**, 87533-35-5; **8** (free base), 87533-62-8; **9**, 87533-36-6; **9** (free base), 87533-63-9; **10**, 87533-37-7; **10** (free base), 87533-64-0; **11**, 87533-38-8; **11** (free base), 87533-65-1; **12**, 87555-26-8; **12** (free base), 87555-28-0; **13**, 87533-39-9; **13** (free base), 87533-66-2; **14**, 87533-40-2; **14** (free base), 87533-67-3; **15**, 87533-41-3; **15** (free base), 87533-68-4; **16**, 87533-42-4; **16** (free base), 87533-69-5; **17**, 87533-43-5; **17** (free base), 81913-31-7; **18**, 87533-44-6; **18** (free base), 87533-70-8; **19**, 87533-46-8; **20**, 87533-71-9; **20** (free base), 87533-72-0; **21**, 87533-47-9; **21** (free base), 87533-73-1; **22**, 87533-48-0; **22** (free base), 87533-74-2; **23**, 87533-49-1; **24**, 87533-50-4; **25**, 87533-51-5; **26**, 35227-79-3; **27**, 87533-52-6; **28**, 87533-53-7; **29**, 87533-54-8; **30**, 87533-55-9; **31**, 79794-86-8; diethyl magnesiomalonate, 87533-56-0; 5-nitroisophthaloyl dichloride, 13438-30-7; diethyl 5-nitroisophthaloylbis(malonate), 87555-27-9; 5-methylisophthaloyl dichloride, 13438-29-4; 4-nitroisophthaloyl dichloride, 13438-30-7; butyllithium, 109-72-8; isophthaldehyde, 626-19-7; 1,3-bis(1-hydroxypentyl)benzene, 87533-57-1; aminoguanidine hydrochloride, 1937-19-5; aminoguanidine methanesulfonate, 87533-58-2; 2-hydrazino-2-imidazoline hydrobromide, 55959-84-7.

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