

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 = N₃; 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(guanyldihydrazone)s. Structure-Activity Relationships among Substituted and Heterocyclic Analogues

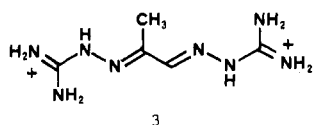
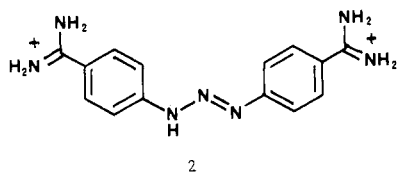
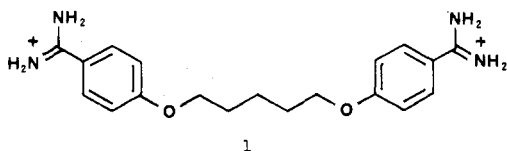
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Based on the antitrypanosomal activity of 1,3-diacetylbenzene bis(guanyldihydrazone) (4) and 2,6-diacetylpyridine bis(guanyldihydrazone) (17), a number of substituted and heterocyclic 1,3-arylene diketone bis(guanyldihydrazone)s were prepared and tested against *Trypanosoma brucei* infections in mice. A wide range of ED₅₀ 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(guanyldihydrazone) (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 guanyldihydrazone side chains was invariably detrimental to activity. Side-chain homologues 1,3-dipentanoylbenzene bis(guanyldihydrazone) 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)



isethionate and diminazene (2) aceturate, are an important class of currently employed antitrypanosomal agents.⁴ Methylglyoxal bis(guanyldihydrazone) (MGG, 3) dihydro-

chloride, 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(guanyldihydrazone)s,⁶ 1,3-diacetylbenzene bis(guanyldihydrazone) (4) and 2,6-diacetylpyridine bis(guanyldihydrazone) (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(guanyldihydrazone) 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.

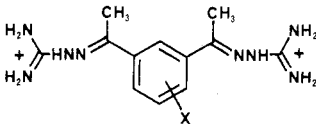
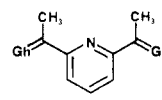
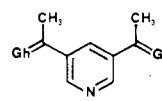
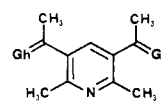
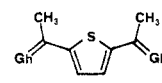
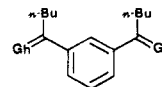
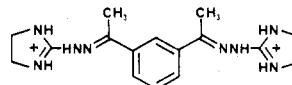
Chemistry. Guanyldihydrazone 4-22 (Table I) were routinely prepared from the corresponding diketone and a 10-20% excess of the appropriate aminoguanidine salt in aqueous ethanol containing a trace of excess acid.

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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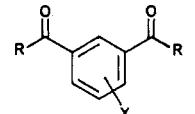
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Table I. Properties of 1,3-Arylene Diketone Bis(guanylhrazone)s

						
no.	X	yield, %	mp, °C	recrystn solvent ^a	formula ^b	
4	H	83	330-333 dec	E-W	C ₁₂ H ₁₈ N ₈ ·2HCl·H ₂ O	
5	5-NH ₂	74	355-360 dec	E-W	C ₁₂ H ₁₉ N ₉ ·2HCl	
6	5-NHCOCH ₃	75	322-325	E-W	C ₁₄ H ₂₁ N ₉ O·2HCl·2H ₂ O	
7	5-OH	62	330-337 dec	E-W	C ₁₂ H ₁₈ N ₈ O·2HCl·2H ₂ O	
8	5-OCH ₃	80	314-316 dec	E-W	C ₁₃ H ₂₀ N ₈ O·2HCl·0.5CH ₃ CH ₂ OH ^c	
9	5-CH ₃	88	345-350 dec	E-W	C ₁₃ H ₂₀ N ₈ ·2HCl·H ₂ O	
10	5-I	70	235-237	E-W	C ₁₂ H ₁₇ N ₈ I·2HCl·0.5H ₂ O	
11	5-NO ₂	92	355-363 dec	E-W	C ₁₂ H ₁₇ N ₈ O ₂ ·2HCl·H ₂ O	
12	5-CCH ₃ =NNHC(=NH)NH ₂ ·HCl	90	345-352 dec	M-W	C ₁₅ H ₂₄ N ₁₂ ·3HCl·H ₂ O	
13	2-NH ₂	77	335-338 dec	E-W	C ₁₂ H ₁₉ N ₉ ·2HCl	
14	2-NO ₂	48	292-295 dec	E-W	C ₁₂ H ₁₇ N ₉ O ₂ ·2HCl	
15	4-NO ₂	79	258-263 dec	E-W	C ₁₂ H ₁₇ N ₉ O ₂ ·2HCl·0.5H ₂ O	
16	4,6-(OH) ₂	85	335-337 dec	E-W	C ₁₂ H ₁₈ N ₈ O ₂ ·2HCl·H ₂ O	
structure ^d						
17		94	360-370 dec	E-W	C ₁₁ H ₁₇ N ₉ ·2HCl·H ₂ O	
18		85	370-372 dec	E-W	C ₁₁ H ₁₇ N ₉ ·2HCl	
19		50	188-190 dec	E-W	C ₁₃ H ₂₁ N ₉ ·2CH ₃ SO ₃ H·H ₂ O	
20		42	340-350 dec	I-W	C ₁₀ H ₁₆ N ₈ S·2HCl·H ₂ O·0.5(CH ₃) ₂ CHOH ^c	
21		73	293-295 dec	E-W	C ₁₈ H ₃₀ N ₈ ·2HCl	
22		58	368-370 dec	E-W	C ₁₆ H ₂₂ N ₈ ·2HBr·1.5H ₂ O	

^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)NH₂·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 ₆ H 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 ₉ IO ₂	C, H	
29	5-NO ₂	CH ₃	99-100	EtOH	C ₁₀ H ₉ NO ₄	C, H, N	
30	4-NO ₂	CH ₃	69-69.5	2-PrOH	C ₁₀ H ₉ NO ₄	C, H, N	
31	H	(CH ₂) ₃ CH 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 CH₂Cl₂. ^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(guanyldiazotone)s 4–22 were assessed by administration to *T. brucei* infected Swiss–Webster mice in a modification⁸ 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(guanyldiazotone) 4. On the basis of ED₅₀ values, the five analogues 5–9 (5-NH₂, 5-NHCOCH₃, 5-OH, 5-OCH₃, and 5-CH₃, 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 guanyldiazotoneethyl group in the trisymmetric analogue 1,3,5-triacetylbenzene tris(guanyldiazotone) (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 guanyldiazotone side chains [2-NH₂, 2-NO₂, 4-NO₂, and 4,6-(OH)₂, respectively], only the 4-nitro derivative 15 had curative activity, about one-fifth that of the parent 4.

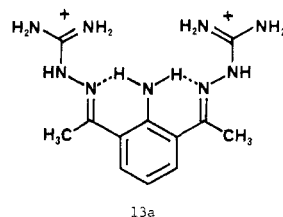
Four heterocyclic congeners of 4 were studied. Of these, only 2,6-diacetylpyridine bis(guanyldiazotone) 17 had equivalent activity. The 3,5-diacetylpyridine analogue 18 and the 2,5-diacetylthiophene derivative 20 were considerably less active than 4 or 17. In compound 19, the 2,6-dimethyl derivative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

An analogue of 4 having longer alkyl side chains, 1,3-dipentanoylbenzene bis(guanyldiazotone) (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.

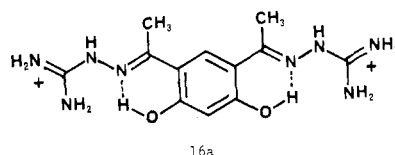
Discussion

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 guanyldiazotone 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 guanyldiazotone moieties.

The detrimental effect of ortho substitution occurred both with H-bonding donor substituents (NH₂ in 13, OH in 16) and non-H-donor groups (NO₂ in 14 and 15 and CH₃ 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 guanyldiazotone side chain. The fact that all ortho interactions studied correlate with loss of activity suggests a require-



13a

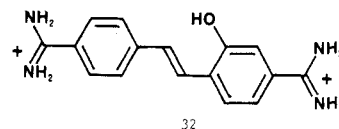


16a

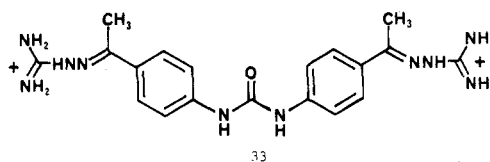
ment of such rotational freedom for optimal antitrypanosomal properties. Energy-dependent uptake of pentamidine (1) is known to occur in trypanosomes.⁹ Conformational flexibility would be an advantage for analogues of 4 if the conformation preferred for an uptake system were different from the conformation 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.

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(guanyldiazotone) (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 trypanosomes¹⁵ and inhibits RNA synthesis in *T. brucei*.¹⁶ On the basis of spectroscopic evidence, it has been proposed that hydroxystilbamidine (32), another bis(benz-



32



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

no.	ED ₅₀ (SD), ^b mg/kg ^b	cures/total mice [toxic deaths] ^a at the following dosages											lower dosages	
		dosages of 150–1.5 mg/kg											mg/kg	c/tot ^c
		150	100	60	40	25	15	10	6	4	2.5	1.5		
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	9/10	20/20	10/10	1.0	8/10
12	0.47 (0.40–0.55)			0/5 [5]	0/5 [4]	4/5 [1]	10/10	10/10	20/20				0.6	20/30
13					0/5 [5]	0/5	0/5						0.4	19/35
14					0/5 [5]	0/5 [3]	0/10						0.25	0/30
15	20 (17–24)	0/5 [5]		0/5 [5]	5/5	10/15	4/15	0/5					0.15	0/10
16			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	16/20	14/25	11/30	0/20	0/10				
20	14 (12–17)		1/5 [4]	5/5	13/15	0/15								
21				0/5 [4]	0/5 [1]	0/5 [1]	0/5 [2]	0/5					1.0	9/10
22	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	0.6	23/25
2 ^d													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(guanylhyazone)	8.5
4	1,3-diacetylbenzene bis(guanylhyazone)	12
2	diminazene	12.5 ^b
32	hydroxystilbamidine	12.5 ^c
1	pentamidine	17
33	4,4'-diacetyldiphenylurea bis(guanylhyazone)	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) trypanocides, 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 trypanocidal 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(guanylhyazone), 4,4'-diacetyldiphenylurea bis(guanylhyazone) (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 guanylhyazone 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 guanylhyazone)s or CDCl₃. 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 N₂, Mg turnings (6.56 g, 0.27 mol) were stirred with EtOH (35 mL) and catalytic CCl₄ (0.5 mL) until rapid H₂ evolution began. Diethyl malonate (43.25 g, 0.27 mol) in Et₂O 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 H₂SO₄. The organic layer was washed with brine and dried (MgSO₄). 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 (CDCl₃) 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 H₂SO₄ (10 mL) and heated at reflux for 3.5 h, at which time CO₂ evolution had ceased. (Larger proportions of water allow decarboxylation 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 SnCl₂·2H₂O (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 K₂CO₃·H₂O (220 g) and ice-water (400 mL). The mixture was extracted with EtOAc (3 × 400 mL). The extracts were washed with 5% NaHCO₃ and brine, dried (MgSO₄), 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 CH₂Cl₂ (15 mL) and treated dropwise with Ac₂O (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 CH₂Cl₂. 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) H₂SO₄ (15 mL) at 0 to 5 °C was treated with solid NaNO₂ (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 HNO₂. The mixture was allowed to warm to room temperature and then was heated to 80 °C over 30 min, at which point N₂ 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 $\text{EtOAc}-\text{C}_6\text{H}_6$ (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 mL, 13.6 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 $\mu\text{L/g}$ of body weight), intraperitoneally once 6 h after infection, at which time bloodstream parasitemia was demonstrable by subinoculation. Un-

treated 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 Bliss²⁹ 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 antipyrene stabilizer found in veterinary Berenil.¹⁵

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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; isophthalaldehyde, 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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