Eur. J. Med. Chem. 36 (2001) 531–538

PII: S0223-5234(01)01250-8/FLA

Original article

Trypanocidal activity of dicationic compounds related to pentamidine

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Received 6 February 2001; revised 11 June 2001; accepted 12 June 2001

Abstract – Eight dicationic compounds related to pentamidine were studied for trypanocidal activity in seven trypanosome isolates. In vitro studies revealed that diamidines are more potent than diimidazolines. For example, **2** (a diamidine) and **4** (a diimidazoline) inhibited the growth of KETRI 243 with IC_{50} values of 2.3 and 900 nM, respectively. Introduction of polar groups into the linker decreased the effectiveness of the compounds against drug-resistant trypanosomes. In compounds with a 2-butene linker between the cationic groups, *trans*-isomers were more potent than *cis*-isomers. The *cis*- and *trans*-buteneamidines cured infection caused by *Trypanosoma brucei brucei* (EATRO Lab 110) and protected mice against infection by *Trypanosoma brucei rhodesiense* isolates, some of which are resistant to diamidines and melarsoprol. © 2001 Éditions scientifiques et médicales Elsevier SAS

antitrypanosomal / dicationic compounds / pentamidine / trypanocidal / trypanosomiasis

1. Introduction

African trypanosomiasis, leishmaniases and Chagas' disease continue to plague tropical and subtropical regions of the world [1]. Chemotherapy for these diseases is still problematic as most of the drugs in use were developed over 40 years ago and suffer from drawbacks ranging from toxicity to the emergence of resistance [1–4]. Aromatic diamidines have shown promise as effective agents for the treatment of protozoal infections [5–7]. A number of molecules belonging to this class of compounds bind to the minor-groove of DNA at AT-rich sites. Interaction of the compounds with DNA minor-groove has been examined at the molecular level using biophysical methods [8–11] and X-ray crystallography [12–15]. Based on the interaction of diamidines with the minor-groove of DNA, it has been postulated that the compounds may exert their biological activity by first binding to DNA, which eventually leads to the inhibition of one or more of several DNA dependent enzymes (e.g. topoisomerases and nucleases) or by the direct inhibition of transcription [10, 16–21]. Indeed, it has been demonstrated that selective binding to kinetoplast DNA plays a role in the anti-microbial action of aromatic diamidines [22]. For example, Agbe and Yielding [23] studied the ability of berenil (an aromatic diamidine) and other drugs to protect mice from death caused by infection with *Trypanosoma brucei* E164 and a dyskinetoplast derivative (Dysk 164). They observed that in all cases death of mice infected with E164 was prevented by drug treatment, whereas death caused by Dysk 164 was not. These findings strongly support the view that the intact kinetoplast plays an essential role in the anti-microbial action of the drugs.

Pentamidine (an aromatic diamidine), is a DNA minor-groove ligand that has been a mainstay for the treatment of early stage African trypanosomiasis for many years but toxicity and the development of resistant strains plagues the drug [1]. There is the need for new and effective agents to treat trypanosomiasis.

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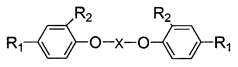
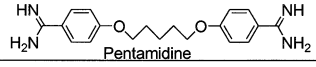
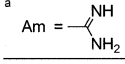
Table I. Structure of pentamidine analogues.



#	R_1[a]	R_2	—X—	mp, °C	Formula[b]
1	Am	H		242	$C_{18}H_{20}N_4O_2.2HCl.1.5H_2O$
2	Am	H		270	$C_{18}H_{20}N_4O_2.2HCl.0.5H_2O$
3	Im	H		270	$C_{22}H_{24}N_4O_2.2HCl.0.25H_2O$
4	Im	H		282	$C_{22}H_{24}N_4O_2.2HCl.0.5H_2O$
5	Am	OCH_3		242	$C_{20}H_{24}N_4O_4.1.8HCl$
6	Im	OCH_3		264	$C_{24}H_{28}N_4O_4.2HCl.0.5H_2O$
7	Am	H		>300	$C_{18}H_{22}N_4O_4.2HCl.1H_2O$
8	Am	H		281	$C_{18}H_{22}N_4O_2.2HCl.0.5H_2O$



Pentamidine

[a] Am =  or Im = 

[b] Elemental analysis (C, H, N, Cl) within ± 0.4% of the theoretical value.

Recently, Keku et al. [24] reported the trypanocidal action of a series of pentamidine related aromatic diamidines. We have reported earlier the synthesis and anti-*Pneumocystis carinii* activity of the pentamidine related aromatic dicationic compounds shown in *table I* [25]. We have also demonstrated that the compounds bind to the minor-groove of the DNA double helix and that the in vitro anti-*P. carinii* action of the compounds correlates with their in vitro DNA binding affinity [26]. As binding of aromatic diamidines to the minor-groove of DNA [10, 16–21] and selective inhibition of kinetoplast DNA synthesis [22, 27] is believed to play a significant role in the anti-microbial action of these compounds, we investigated the trypanocidal activity of the aromatic dicationic compounds shown in *table I*.

2. Chemistry

Compounds **1**–**8** were synthesized by following our methods described earlier as shown in *figure 1* [25]. Briefly, dicyano **11** was synthesized via the Williamson ether synthesis. Compound **11** was transformed to the desired amidine or imidazoline via the Pinner reaction followed by refluxing the resulting imidate **12** in methanolic ammonia or ethylenediamine. High-performance liquid chromatography, elemental analysis, and proton magnetic resonance spectrometry established the purity of each compound. The structures of the compounds along with their physical data are shown in *table I*.

3. Results and discussion

3.1. Structure–activity relationship studies

The compounds were screened in vitro for anti-trypanosomal activity against one *Trypanosoma brucei brucei* pentamidine-sensitive strain (EATRO Lab 110) and

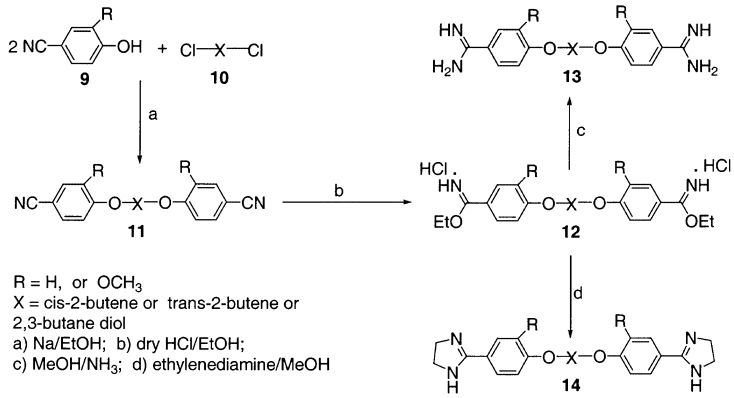


Figure 1. Synthesis of compounds **1**–**8**.

Table II. In vitro anti-trypanosomal activity and DNA binding affinity of pentamidine and its analogues **1**–**8**.

Compound	IC_{50} (nM)					
	EATRO Lab 110	KETRI			ΔT_m	
		243	269	243As-10-3	CT [a]	AT [b]
cis-Buteneamidine (**1**)	7.2	7.8	14.5	8.8	11.0	31.5
trans-Buteneamidine (**2**)	2.3	2.2	2.5	2.4	7.0	23.3
3	26.5	12.2	17.0	88.0	9.3	28.9
4	69.5	900.0	62.0	77.0	7.9	20.8
5	45.0	62.0	71.9	18.9	ND [c]	ND
6	67.0	50.0	55.0	44.0	ND	ND
7	7.3	22.4	54.0	230.0	ND	ND
Butamidine **8**	6.7	6.0	7.2	6.8	8.3	20.4
Pentamidine (**9**)	0.8	3.1	3.2	5.0	10.0	22.9

[a] CT is sonicated calf thymus DNA.
[b] AT is sonicated poly(dA)poly(dT) homopolymer.
[c] ND, not determined.

three drug resistant clinical isolates of *Trypanosoma brucei rhodesiense* (K243, K269, and K243As-10-3). The results are shown in *table II*. *trans*-Buteneamidine (**2**) was the most potent agent of the series. It inhibited the growth of the trypanosome isolates at IC_{50} values of 2.5 nM or less. Pentamidine was about 3-fold more effective than the *trans*-buteneamidine against the pentamidine-sensitive isolate (EATRO Lab 110) but *trans*-buteneamidine was as potent (vs. KETRI 243 and KETRI 269) or slightly more potent (vs. KETRI 243As-10-3) than pentamidine in inhibiting growth of the pentamidine-resistant isolates. In compounds with amidine groups, anti-trypanosomal activity was influenced by the geometry of the linker. For instance, *trans*-buteneamidine was at least 3-fold more potent than *cis*-buteneamidine in inhibiting the growth of all the trypanosome isolates studied. In the absence of methoxy substituents (as in compounds **1**–**4**), amidine derivatives were more potent than imidazoline derivatives. The difference in the anti-trypanosomal activity of such compounds was more pronounced in compounds with *trans* geometry (**2** vs. **4**) than those with *cis* geometry (**1** vs. **3**). For example, *trans*-buteneamidine was at least 25-fold more potent against all the trypanosome isolates compared with its imidazoline counterpart **4**. *cis*-Buteneamidine (**1**) however, was at the most only 10-fold more potent against all the trypanosome isolates compared with its imidazoline counterpart **3**. This difference in anti-trypanosomal activity between amidines and imidazolines was particularly pronounced with the drug-resistant isolate K243 in which *trans*-buteneamidine (**2**) was over 400-fold more potent than its imidazoline counterpart (compound **4**). Introduction of methoxy groups *ortho* to the bridge was more detrimental to the anti-trypanosomal action of amidine derivatives than the imidazoline derivatives. For example, compound **5** was between 8- and 29-fold less active than compound **2** depending on the trypanosome isolate. A similar methoxy substitution in the imidazoline derivative **4** to give compound **6** did not result in a concomitant drastic decrease in the anti-trypanosomal action. On the contrary, 18-fold increase in activity against KETRI 243 was observed. Compound **7**, which is a 2,3-butanediol analogue of butamidine (**8**), was equipotent to **8** in inhibiting the growth of pentamidine-sensitive trypanosomes (EATRO Lab 110). It was however, 4-, 8-, and 34-fold less potent than butamidine against the three drug-resistant trypanosome isolates K243, K269, and K243As-10-3, respectively. The inequity in the anti-trypanosomal action between butamidine and its 2,3-butanediol analogue **7** against the pentamidine-resistant isolates may be due in part to the difference in the lipophilicity of the compounds. Butamidine (ca. $\log P = 0.91$), being more lipophilic may penetrate the parasites better (via passive diffusion) than the more polar analogue **7** (ca. $\log P = -1.81$). The $\log P$ values were calculated with the PROLOG P program using the CDR segment database (CompuDrug Inc., Erno, Hungary).

3.2. In vivo anti-trypanosomal activity

Geometric isomers **1** and **2** demonstrated good in vitro activity against all of the four trypanosome isolates

studied (*table I*). The compounds were therefore selected for in vivo studies in mouse model infections using *T. brucei brucei* EATRO Lab 110 and *T. brucei rhodesiense* KETRI isolates (K243, K243As-10-3, K269, K2002, K2538, and K2545). The compounds were administered intraperitoneally (i.p.) as single daily injections over three days and the results are shown in *tables III to V*. The compounds were curative versus five of the seven isolates studied (EATRO Lab 110, KETRI 2002, KETRI 2538, KETRI 2545, and KETRI 269). At doses ranging from 2.5 to 25 mg kg^{-1} per day, *cis*- and *trans*-buteneamidines cured 40–100% of the animals

Table III. In vivo trypanocidal effects of pentamidine (Pent.), *cis*-buteneamidine (**1**) and *trans*-buteneamidine (**2**) against *T. brucei brucei* Lab 110 and *T. brucei rhodesiense* KETRI 269.

Microbe [a]	*T. brucei brucei* Lab 110			Microbe [a]	*T. brucei rhodesiense* KETRI 269		
Dose (mg kg^{-1})	# Cured/total (MSD) [b]			Dose (mg kg^{-1})	# Cured/total (MSD) [b]		
	Pent.	**1**	**2**		Pent.	**1**	**2**
1.0	5/5 (–) [c]	0/5 (3.8)	0/5 (8.4)	1.0	0/5 (14.8)	0/5 (12.2)	0/5 (11.6)
2.5	5/5 (–)	2/5 (15.7)	5/5 (–)	2.5	ND	2/5 (27)	0/5 (19.6)
5.0	5/5 (–)	5/5 (–)	5/5 (–)	5.0	4/5 (34.0)	3/5 (32.0)	3/5 (26.0)
10.0	ND [d]	5/5 (–)	5/5 (–)	10.0	4/5 (21.0)	3/5 (27.0)	5/5 (–)
25.0	ND	5/5 (–)	5/5 (–)	25.0	5/5 (–)	5/5 (–)	5/5 (–)
5.0 (1×) [e]	ND	ND	5/5 (–)	5.0 (1×) [e]	ND	ND	ND
10.0 (1×) [e]	ND	ND	4/5 (8.0)	10.0 (1×) [e]	ND	ND	ND
25.0 (1×) [e]	ND	5/5 (–)	9/10 (5.0)	25.0 (1×) [e]	ND	4/5 (10.0)	2/5 (10.3)

[a] Mice were infected (2.5×10^5 parasites) and the infections were allowed to progress 24 h before treatment began. Compounds were dissolved in distilled water (vehicle) and injected i.p. once a day for 3 days. Mice treated with vehicle as control all died with MSD values ranging between 3.2 and 11.4 days.
[b] MSD is the mean survival time (in days) of animals dying of trypanosomiasis exclusive of cured animals. Animals surviving >30 days beyond the death of the last control with no parasites in tail blood smears were considered cured. Five mice were studied at each drug dose with MSD values for each study group shown in parenthesis.
[c] '–' means all the animals were cured as defined above.
[d] ND, not determined.
[e] Only one i.p. injection of the compound was administered.

Table IV. In vivo trypanocidal effects of pentamidine (Pent.), *cis*-buteneamidine (**1**) and *trans*-buteneamidine (**2**) against *T. brucei rhodesiense* KETRI 2002 and *T. brucei rhodesiense* KETRI 2538.

Microbe [a]	*T. brucei rhodesiense* KETRI 2002			Microbe [a]	*T. brucei rhodesiense* KETRI 2538		
Dose (mg kg^{-1})	# Cured/total (MSD) [b]			Dose (mg kg^{-1})	# Cured/total (MSD) [b]		
	Pent.	**1**	**2**		Pent.	**1**	**2**
1.0	5/5 (–) [c]	0/5 (17.4)	5/5 (–)	1.0	5/5 (–) [c]	0/5 (4.6)	5/5 (–)
2.5	ND [d]	2/5 (32.3)	5/5 (–)	2.5	5/5 (–)	2/5 (12.7)	5/5 (–)
5.0	5/5 (–)	5/5 (–)	5/5 (–)	5.0	5/5 (–)	5/5 (–)	5/5 (–)
10.0	5/5 (–)	5/5 (–)	5/5 (–)	10.0	ND	5/5 (–)	ND
25.0	5/5 (–)	4/5 (6.0)	5/5 (–)	25.0	ND	5/5 (–)	ND

[a] Mice were infected (2.5×10^5 parasites) and the infections were allowed to progress 24 h before treatment began. Compounds were dissolved in distilled water (vehicle) and injected i.p. once a day for 3 days. Mice treated with vehicle as control all died with MSD values ranging between 3.2 and 11.4 days.
[b] MSD is the mean survival time (in days) of animals dying of trypanosomiasis exclusive of cured animals. Animals surviving >30 days beyond the death of the last control with no parasites in tail blood smears were considered cured. Five mice were studied at each drug dose with MSD values for each study group shown in parenthesis.
[c] '–' means all the animals were cured as defined above.
[d] ND, not determined.

Table V. In vivo trypanocidal effects of pentamidine (Pent.), *cis*-buteneamidine (**1**) and *trans*-buteneamidine (**2**) against *T. brucei rhodesiense* KETRI 2545 and *T. brucei rhodesiense* KETRI 243.

Microbe [a]	*T. brucei rhodesiense* KETRI 2545			Microbe [a]	*T. brucei rhodesiense* KETRI 243		
Dose (mg kg^{-1})	# Cured/total (MSD) [b]			Dose (mg kg^{-1})	# Cured/total (MSD) [b]		
	Pent.	**1**	**2**		Pent.	**1**	**2**
1.0	0/5 (22.2)	0/5 (13.8)	0/5 (12.0)	1.0	ND	0/5 (10.2)	ND
2.5	4/5 (23.0)	ND	ND	2.5	ND	0/5 (9.6)	ND
5.0	5/5 (–) [c]	4/5 (51.0)	4/5 (23.0)	5.0	0/5 (12.6)	0/5 (10.0)	0/5 (13.0)
10.0	5/5 (–)	4/5 (35.0)	5/5 (–)	10.0	0/5 (13.4)	0/5 (10.0)	1/5 (20.5)
25.0	ND [d]	4/5 (14.0)	5/5 (–)	25.0	0/5 (16.6)	0/5 (13.8)	1/5 (25.3)

[a] Mice were infected (2.5×10^5 parasites) and the infections were allowed to progress 24 h before treatment began. Compounds were dissolved in distilled water (vehicle) and injected i.p. once a day for 3 days. Mice treated with vehicle as control all died with MSD values ranging between 3.2 and 11.4 days.
[b] MSD is the mean survival time (in days) of animals dying of trypanosomiasis exclusive of cured animals. Animals surviving >30 days beyond the death of the last control with no parasites in tail blood smears were considered cured. Five mice were studied at each drug dose with MSD values for each study group shown in parenthesis.
[c] '–' means all the animals were cured as defined above.
[d] ND, not determined.

infected with EATRO Lab 110 (*table III*). *trans*-Buteneamidine was also curative at single injections of 5, 10, and 25 mg kg^{-1} doses (80% cure) versus Lab 110. The compounds were also curative against infections caused by KETRI 269, which are DFMO and pentamidine resistant. At 10 mg kg^{-1}, the *trans*-isomer (**2**) was more effective than pentamidine and the *cis*-isomer (**1**) against KETRI 269 (*table III*). The *trans*-isomer also effectively cured infections caused by the DFMO-refractory isolate KETRI 2002 (*table IV*) and KETRI 2538 (*table IV*), which is sensitive to standard trypanocides. The *trans*-isomer was more effective than the *cis*-isomer against these isolates and it displayed a dose–response profile identical to that of pentamidine (*table IV*). The *trans*-isomer was also a better agent than the *cis*-isomer at treating infection caused by KETRI 2545 (*table V*). The *cis*-isomer was not active against arsenical and pentamidine resistant KETRI 243. The *trans*-isomer on the other hand demonstrated some activity (20% cures and 2- to 2.5-fold MSD prolongation compared with the *cis*-isomer) against KETRI 243 (*table V*).

4. Conclusions

The in vitro studies suggest that aromatic diamidines are better trypanocidal agents than their diimidazoline congeners. In model infections, both *cis*- and *trans*-buteneamidines cured mice infected with a pentamidine-sensitive isolate (EATRO Lab 110) and were curative versus infections caused by the two drug-resistant isolates (KETRI 269 and KETRI 2002). The *trans*-isomer prolonged the life of mice and produced partial cures of mice infected with a pentamidine and melarsen resistant isolate (KETRI 243), but none of the two isomers was active against KETRI 243 As-10-3, which is completely refractory to melarsen and pentamidine. The anti-trypanosomal action of the compounds suggests that further structure–activity relationship studies involving modification of the linker between the dicationic compounds is warranted.

5. Experimental

5.1. Chemistry

The compounds tested were synthesized as described by us earlier [25]. Briefly, the appropriately substituted 4-cyanophenol **9** was reacted with the appropriate dichloro linker **10** to give the corresponding dicyano derivative **11** (*figure 1*). Treatment of dicyano **11** in dry toluene–EtOH mixture with dry HCl gas gave imidate hydrochloride **12**. Reaction of **12** with either MeOH–NH_3 or ethylenediamine gave the corresponding di-

amidine or diimidazoline derivative, respectively. Elemental analyses were within ±0.4% of the theoretical values.

5.1.1. cis-1,4-Bis(4-amidinophenoxy)but-2-ene ***(1)***

M.p. (dec.): 242 °C. IR (Nujol, cm^{-1}): ν(NH) 3325, ν(C=C) 1671, ν(C=N) 1626, ν(C–O–C) 1288, ν(C–O–C) 1031, ν(C=C) 848. ^{1}H-NMR (D_2O): δ 4.93 (br d, 4H, –OCH_2CH=CHCH_2O–), 6.11 (t, 2H, –CH=CH–), 7.16 (d, 4H, aromatic protons), 7.81 (d, 4H, aromatic protons). Anal. $C_{18}H_{20}N_4O_2 \cdot 2HCl \cdot 1.5H_2O$ (C, H, N, O, Cl).

5.1.2. trans-1,4-Bis(4-amidinophenoxy)but-2-ene ***(2)***

M.p. (dec.): 270 °C. IR (Nujol, cm^{-1}): ν(NH) 3298, ν(C=C) 1673, ν(C=N) 1613, ν(C–O–C) 1255, ν(C–O–C) 1080, ν(C=C) 848. ^{1}H-NMR (D_2O): δ 4.82 (br d, 4H, –OCH_2CH=CHCH_2O–), 6.15 (t, 2H, –CH=CH–), 7.16 (d, 4H, aromatic protons), 7.76 (d, 4H, aromatic protons). Anal. $C_{18}H_{20}N_4O_2 \cdot 2HCl \cdot 0.5H_2O$ (C, H, N, O, Cl).

5.1.3. cis-1,4-Bis(4-imidazolinophenoxy)but-2-ene ***(3)***

M.p. (dec.): 270 °C. IR (Nujol, cm^{-1}): ν(NH) 3405, ν(C=N) 1626, ν(C–O–C) 1250, ν(C–O–C) 1047, ν(C=C) 843. ^{1}H-NMR (D_2O): δ 4.08 (s, 8H, –NCH_2CH_2N–), 4.87 (br d, 4H, –OCH_2CH=CHCH_2O–), 6.07 (t, 2H, –CH=CH–), 7.14 (d, 4H, aromatic protons), 7.77 (d, 4H, aromatic protons). Anal. $C_{22}H_{24}N_4O_2 \cdot 2HCl \cdot 0.25H_2O$ (C, H, N, O, Cl).

5.1.4. trans-1,4-Bis(4-imidazolinophenoxy)but-2-ene ***(4)***

M.p. (dec.): 282 °C. IR (Nujol, cm^{-1}): ν(NH) 3086, ν(C=N) 1617, ν(C–O–C) 1244, ν(C–O–C) 1089, ν(C=C) 839. ^{1}H-NMR (D_2O): δ 4.06 (s, 8H, –NCH_2CH_2N–), 4.77 (br d, 4H, –OCH_2CH=CHCH_2O–), 6.11 (t, 2H, –CH=CH–), 7.13 (d, 4H, aromatic protons), 7.72 (d, 4H, aromatic protons). Anal. $C_{22}H_{24}N_4O_2 \cdot 2HCl \cdot 0.5H_2O$ (C, H, N, O, Cl).

5.1.5. trans-1,4-Bis(4-amidino-2-methoxyphenoxy)-but-2-ene ***(5)***

M.p. (dec.): 242 °C. ^{1}H-NMR (DMSO-d_6): δ 3.88 (s, 6H, –OCH_3), 4.73 (br d, 4H, –OCH_2CH=CHCH_2O–), 6.12 (t, 2H, –CH=CH–), 7.20 (d, 2H, aromatic protons), 7.52 (s, 2H, aromatic protons), 7.55 (m, 2H, aromatic protons), 9.06 (s, 4H, protonated amidine), 9.32 (s, 4H, protonated amidine). Anal. $C_{20}H_{24}N_4O_4 \cdot 1.8HCl$ (C, H, N, O, Cl).

5.1.6. trans-1,4-Bis(4-imidazolino-2-methoxyphenoxy)-but-2-ene ***(6)***

M.p. (dec.): 264 °C. ^{1}H-NMR (DMSO-d_6): δ 3.85 (s, 6H, –OCH_3), 3.96 (s, 8H, –NCH_2CH_2N–), 4.88 (br d, 4H, –OCH_2CH=CHCH_2O–), 5.91 (t, 2H, –CH=CH–), 7.20 (d, 2H, aromatic protons), 7.75 (dd, 2H, aromatic protons), 7.81 (d, 2H, aromatic protons), 10.76 (s, 4H, protonated imidazoline) Anal. $C_{24}H_{28}N_4O_2 \cdot 2HCl \cdot 0.5H_2O$ (C, H, N, O, Cl).

5.1.7. 1,4-Bis(4-amidinophenoxy)-2,3-butanediol ***(7)***

M.p. (dec.): >300 °C. ^{1}H-NMR (DMSO-d_6): δ 3.43 (br s, 2H, OH), 3.98 (m, 2H, –CH_2CH(OH)-CH(OH)CH_2–), 4.10 (m, 2H, –CH_2CH(OH)CH(OH)-CH_2–), 4.22 (m, 2H, –CH_2CH(OH)CH(OH)CH_2–), 7.16 (d, 4H, aromatic protons), 7.87 (d, 4H, aromatic protons), 9.09 (s, 4H, protonated amidine), 9.23 (s, 4H, protonated amidine). Anal. $C_{18}H_{22}N_4O_2 \cdot 2HCl \cdot 1H_2O$ (C, H, N, O, Cl).

5.1.8. 1,4-Bis(4-amidinophenoxy)butane ***(8)***

M.p. (dec.): 281 °C. ^{1}H-NMR (DMSO-d_6): δ 1.89 (s, 4H, –OCH$_2$CH_2CH_2CH$_2$O–), 4.15 (s, 4H, –OCH_2CH$_2$-CH$_2$CH_2O–), 7.13 (d, 4H, aromatic protons), 7.87 (d, 4H, aromatic protons), 9.12 (s, 4H, protonated amidine), 9.31 (s, 4H, protonated amidine). Anal. $C_{18}H_{22}N_4O_2 \cdot 2HCl \cdot 0.5H_2O$ (C, H, N, O, Cl).

5.2. Pharmacology

T. brucei brucei Lab 110 EATRO strain (pentamidine-sensitive) and clinical isolates of *T. brucei rhodesiense* were used in this study. *T. brucei rhodesiense* isolates were obtained from A.R. Njogu of the Kenya Trypanosomiasis Research Institute (KETRI: Muguga, Kenya). These included KETRI 243 (DFMO, melarsoprol, pentamidine, and berenil resistant); KETRI 243As-10-3 which is a cloned subpopulation of KETRI 243 and is refractory to arsenicals and aromatic diamidines such as berenil and pentamidine; KETRI 269 (DFMO and pentamidine resistant); KETRI 2002 (DFMO resistant); KETRI 2545; and KETRI 2538 [28].

5.2.1. In vitro studies

The compounds were tested against trypanosome isolates grown as blood forms in HMI-18 medium [29] containing 20% horse serum in 24-well microplates at 37 °C. Wells were inoculated with 1×10^5 trypanosomes.

The compounds were diluted in the medium at the appropriate concentration and replaced daily. Cell counts were made daily with a Coulter Counter, Model Z1 (Beckman Coulter, Miami, Florida, USA). Cells were diluted with Isoton I buffer (Beckman Coulter) and the aperture was standardised at 5.14 μm. Background checks were performed daily on 1:10 dilutions of medium. Counts, normalisation, coincident counts, etc. were accounted for by the standardised Coulter analysis program. Occasionally, hemocytometer counts were carried out as a check on the validity of the Coulter program.

IC_{50} values were determined after 48 h from semi-log plots and the values are the result of duplicate determinations. Initially a broad concentration curve was used and then a close concentration curve from which IC_{50} values were determined. Assays were carried out in duplicate and each point was the average of the two. Control cells grew to 5×10^6 mL^{-1}.

5.2.2. In vivo studies

Female Swiss-Webster mice (weight, 20 g) were infected (intraperitoneally) with 2.5×10^5 trypanosomes from rat blood, and the infection was allowed to develop for 24 h before the drug treatment. Groups of five mice each were injected (i.p.) with each drug concentration. All the experiments included a group of untreated controls. Untreated mice died 5–13 days after the infection, depending on the isolate. Parasitemia of animals dying of infection averaged $0.5–1.0\times10^9$ mL^{-1} of blood. These infections are highly predictable and daily counts were not carried out because of the labour involved. The procedures used are standard for our laboratory [28]. Animals were monitored weekly for parasites in tail vein blood smears. Mice were considered cured if they survived for more than 30 days after the death of the last control with no parasites in tail vein blood smears. MSD (mean survival in days) was also recorded for each group. It is the average time of survival of the animals in the group, exclusive of cured animals.

Acknowledgements

Part of this study was funded by NIH grant 1R15 AI/OD 39683 to I.O.D. and a grant from the World Health Organization/World Bank/United Nations Development Programme Special Programme for Tropical Disease Research to C.J.B (# 950504).

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