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In Vitro Trypanocidal Activities of New *S*-Adenosylmethionine Decarboxylase Inhibitors

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A series of novel aromatic derivatives based on the structure of methylglyoxal bis(guanylhydrazone) (MGBG) was examined for in vitro antitrypanosomal activities and cytotoxicities for human cells. One-third of the compounds tested showed trypanocidal activity at concentrations below 0.5 μM after an incubation period of 72 h. Structure-activity analysis revealed that bicyclic compounds with homocyclic rings and unmodified termini were the most active compounds. Results obtained in three laboratories employing different methods and trypanosome populations consistently ranked compound CGP 40215A highest. This compound had a 50% inhibitory concentration of 0.0045 μM for *Trypanosoma brucei rhodesiense*, was also active against other trypanosome species, including a multidrug-resistant *Trypanosoma brucei brucei*, and was significantly less toxic than other compounds tested for a human adenocarcinoma cell line, with a 50% inhibitory concentration of 1.14 mM. The effect of CGP 40215A was time and dose dependent, and low concentrations of the compound required exposure times of >2 days to exert trypanocidal activity. Compounds were inactive against *Leishmania donovani* and *Trypanosoma cruzi* amastigotes in murine macrophages in vitro.

The World Health Organization lists three diseases caused by kinetoplastids as being of primary importance in tropical and subtropical regions of the world: African trypanosomiasis, the leishmaniases, and Chagas' disease (34). Chemotherapy for clinical disease and prophylaxis in animals remains a primary concern, since most available drugs have been in use for more than 40 years and are toxic while resistance to most of the primary agents, including the diamidines diminazene (Berenil) and pentamidine and the arsenical melarsoprol (Arsobal), as well as the antimonials and benznidazole, has increased (6, 31, 33, 34).

Polyamine biosynthesis has long been regarded as a potential selective therapeutic target in tumor cells as well as in rapidly dividing parasites (3, 8, 22, 25). Extensive biochemical and metabolic studies have concentrated on the properties of the two regulatory enzymes of the pathway, ornithine decarboxylase and *S*-adenosylmethionine (AdoMet) decarboxylase, and extensive synthetic programs have been initiated to develop inhibitors (24, 28).

The first effective inhibitor of AdoMet decarboxylase was MGBG [methylglyoxal bis(guanylhydrazone)], which proved to be clinically active for human leukemia (19). The compound showed a trypanocidal effect against *Trypanosoma brucei* procyclic forms in vitro; however, it failed to cure infected mice (7). It was also inactive against *Leishmania donovani* amastigotes in murine macrophages in vitro (20). Related drawbacks of MGBG concern its lack of specificity due to its interference with mitochondrial function (23) and its toxicity (32).

A recent synthesis program has produced a series of aromatic MGBG analogs which are AdoMet decarboxylase inhibitors having low levels of toxicity and high levels of antitumor activity (26, 27, 30). In this report we describe the antitrypanosomal effects of AdoMet decarboxylase inhibitors for different species of African trypanosomes as well as their toxicity for a human adenocarcinoma cell line.

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MATERIALS AND METHODS

Trypanosome strains. *Trypanosoma brucei rhodesiense* STIB 900 is a cloned population isolated in 1982 from a patient in Tanzania. *Trypanosoma brucei gambiense* STIB 930 is a derivative of TH1/78E(031), which was isolated in 1978 from a patient in Ivory Coast (18). *Trypanosoma brucei brucei* STIB 950 is a cloned population isolated in 1985 from a bovine in Somalia; it shows drug resistance to diminazene, isometamidium, and quinapyramine (13). *Trypanosoma congolense* STIB 910 is a cloned derivative of STIB 249 originally isolated in 1971 from a lion in Tanzania (10). The histories of *T. brucei brucei* S427 and the *T. brucei rhodesiense* isolates KETRI 243 (melarsoprol, pentamidine, and diminazene resistant), 269 (pentamidine resistant), and 2538 were described by Bacchi et al. (1, 4).

Human cell line. The human cell line HT-29 derived from an isolated primary case of adenocarcinoma in 1964 was obtained from the American Type Culture Collection (reference no. HTB 38).

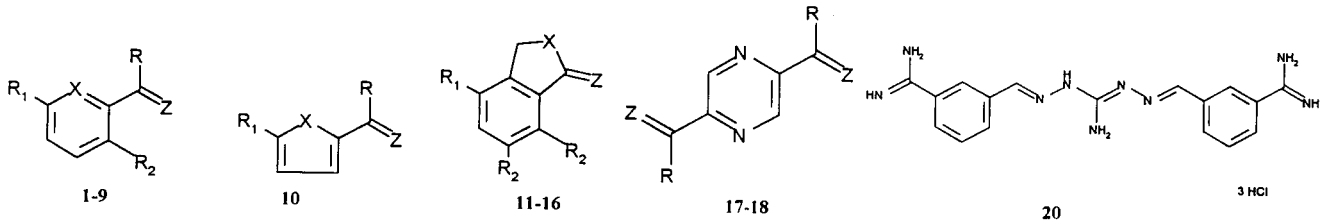
Culture media used for growth of trypanosome strains. All strains used were adapted to grow under axenic culture conditions according to Baltz et al. (5). The medium for *T. brucei brucei* and *T. brucei rhodesiense* strains contained 10% heat-inactivated horse serum or heat-inactivated fetal bovine serum. For *T. brucei gambiense* the medium was supplemented with 10% human serum plus 10% heat-inactivated fetal bovine serum instead of horse serum. *T. congolense* was cultured in Iscove's modified Dulbecco's medium with various supplements, including 20% heat-inactivated caprine serum, according to the method of Kaminsky et al. (12).

Stock cultures were kept in either T-25 flasks or 24-well tissue culture plates at 37°C (for *T. congolense* only, at 34°C) in an atmosphere of 5% CO_2 in air.

Determination of in vitro antitrypanosomal and antileishmanial activities. Different methods to determine the antitrypanosomal activities of inhibitors for trypanosome species which have slightly different growth requirements were used in laboratories in Basel, London, and New York.

Fluorescence assay used in Basel. Fifty percent inhibitory concentrations (IC_{50}s) for the Swiss Tropical Institute isolates were determined in the laboratory in Basel. Briefly, the fluorochrome 2′,7′-bis(carboxyethyl)-5(6)-carboxyfluores-

TABLE 1. Structures and antitrypanosomal activities of compounds 1 to 20



Compound no.	CGP no.	X	R	R_1	R_2	Z	Molecular weight	Formula	*T. brucei rhodesiense* STIB 900	
									MIC (μM)	IC_{50} (μM)
1	36343A	CH	H	$COO(CH_2)_2NH(CH_2)_2OH$	H	$=NNH(C=NH)NH_2$	366.3	$C_{13}H_{19}N_5O_3 \cdot 2HCl$	132	35.6
2	46774A	N	H	4,5-dihydro-imidazolyl-2	H	$=NNH(C=NH)NH_2$	304.2	$C_{10}H_{13}N_7$ 2HCl	329	4.24
3	47936A	CH	CH_3	$(C=NH)NH_2$	OH	$=NNH(C=NH)NH_2$	307.2	$C_{10}H_{14}N_6O$ 2HCl	36.1	14.6
4	48030A	CH	C_2H_5	$(C=NH)NH_2$	H	$=NNH(C=NH)NH_2$	330.4	$C_{11}H_{16}N_6$ H_2SO_4	33.6	10.9
5	54764A	CH	H	$(C=NH)NH_2$	H	$=NNCH_3(C=NH)NH_2$	316.3	$C_{10}H_{14}N_6$ H_2SO_4	23.4	1.9
6	35753A	CH	H	$(C=NH)NH_2$	H	$=NNH(C=NOH)NH_2$	293.2	$C_9H_{12}N_6O$ 2HCl	114	14.4
7	46771A	N	H	$(C=NH)NH_2$	H	$=NNH(C=NOH)NH_2$	294.1	$C_8H_{11}N_7O$ 2HCl	87.1	18.3
8	40214A	CH	H	$(C=NH)NH_2$	H	$=NNH(C=NNH_2)NH_2$	292.2	$C_9H_{13}N_7$ 2HCl	1.3	0.116
9	46987A	N	H	$(C=NH)NH_2$	H	$=NNH(C=NNH_2)NH_2$	293.2	$C_8H_{12}N_8$ 2HCl	74.4	23.5
10	49776A	S	H	$(C=NH)NH_2$	H	$=NNH(C=NH)NH_2$	283.2	$C_7H_{10}N_6S$ 2HCl	2.67	0.13
11	48664A	CH_2		$(C=NH)NH_2$	H	$=NNH(C=NH)NH_2$	321.9	$C_{11}H_{14}N_6 \cdot 2HCl \cdot H_2O$	49.2	0.49
12	50019A	$(CH_2)_2$		$(C=NH)NH_2$	H	$=NNH(C=NH)NH_2$	317.2	$C_{12}H_{16}N_6$ 2HCl	2.35	0.61
13	52043A	CH_2		$(C=NH)NH_2$	OCH_3	$=NNH(C=NH)NH_2$	363.2	$C_{13}H_{18}N_6O_2$ 2HCl	1.4	0.3
14	53391A	CH_2		$(C=O)NH_2$	H	$=NNH(C=NH)NH_2$	267.7	$C_{11}H_{13}N_5O$ 2HCl	116	36.9
15	53392A	CH_2		$(C=NH)NH_2$	H	$=NNH(C=O)NH_2$	267.7	$C_{11}H_{13}N_5O$ 2HCl	174	81.9
16	56416A	CH_2		$(C=NOH)NH_2$	H	$=NNH(C=NH)NH_2$	319.2	$C_{11}H_{14}N_6O$ 2HCl	313	113
17	48104A	CH_3				$=NNH(C=NH)NH_2$	349.2	$C_{10}H_{16}N_6O$ 2HCl	31	12.3
18	48695A	C_2H_5				$=NNH(C=NH)NH_2$	377.3	$C_{12}H_{20}N_{10}$ 2HCl	133	35.8
19	48647A			Diethylglyoxal-bis-guanyl-hydrazone			324.4	$C_8H_{18}N_8$ H_2SO_4	34.2	1.39
20	40215A						512.8	$C_{17}H_{19}N_9$ 3HCl $3H_2O$	0.093	0.0045

cein-pentaacetoxy-methylester (BCECF-AM) was added to the cultures subsequent to a 72-h drug exposure (21). In viable cells, the nonfluorescent BCECF-AM is cleaved by unspecific esterases into fluorescent products. After incubation for 1 h, fluorescence was quantified with a CytoFluor (model 2300; Millipore, Bedford, Mass.). The IC_{50}s of fluorescence were then calculated. MICs were determined microscopically in the same assay after 72 h of drug exposure and prior to the addition of the fluorochrome. MIC was defined as the lowest concentration at which no trypanosome with normal morphology or motility could be found.

Growth inhibition assay for *T. brucei gambiense* used in Basel. The fluorescence assay could not be employed for the determination of drug sensitivities for *T. brucei gambiense* because of a high-level unspecific esterase activity in human serum which is required for growth of this trypanosome species. Therefore, a previously described growth inhibition assay was used (16). The assay was modified such that *T. brucei gambiense* populations were exposed to the drug for 72 h.

Sulforhodamine assay for *T. congolense* used in Basel. The sensitivity of *T. congolense* was determined by the colorimetric drug sensitivity assay as described by Kaminsky and Wasike (15).

Growth inhibition assay used in London. Trypomastigote bloodstream forms cultured in HMI-9 medium (11) were diluted to 10^5 cells per ml, and 100 μl was dispensed into wells of a microtiter plate. Test compounds diluted in HMI-9 medium were added to other 100-μl volumes to give final drug concentrations in triplicate. Cultures were incubated at 37°C in a 5% CO_2–air mixture for 48 h, the number of cells in each well was counted in a ZM Coulter counter with a 70-μm aperture, and IC_{50}s were determined.

Growth inhibition assay used in New York. Trypomastigote bloodstream forms were cultured in HMI-18 medium (11) with 20% horse serum instead of synthetic serum and hypoxanthine at a concentration of 1 μM instead of 10 μM (2). Drug studies were done in duplicate in 24-well plates (1 ml per well) with final inhibitor concentrations of 0.1, 1, 10, 25, and 100 μM. After 48 h, the parasite number was determined in a Z1 Coulter counter and the IC_{50}s were determined.

***L. donovani* and *Trypanosoma cruzi* assays.** Mouse peritoneal macrophages were isolated and maintained in Labtek 8 chambers in RPMI 1640 medium with 10% heat-inactivated fetal bovine serum at 37°C in a 5% CO_2–air mixture. *L. donovani* amastigotes were isolated from spleens of hamsters and used to infect the macrophages. Infected cultures were maintained in media containing the inhibitors in threefold dilutions for 5 days according to the method of Neal and Croft (20). The media were replaced twice with fresh media containing a drug during this period. After 5 days, slides were methanol fixed and Giemsa stained and the proportion of infected macrophages in each well was determined. IC_{50}s were determined by linear regression analysis.

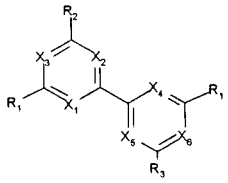
For *T. cruzi*, macrophages were infected with trypomastigotes from the supernatant of L6 myoblast cultures at a ratio of five parasites per macrophage and incubated at 37°C in a 5% CO_2–air mixture for 24 h. Infected macrophage cultures were then exposed to threefold serial dilutions of an inhibitor in quadruplicate samples. After 3 days, slides were fixed, stained, and examined, and IC_{50}s were determined as described above.

Determination of cytotoxicity for HT-29 cells. The sulforhodamine assay, used for *T. congolense*, was also applied to human cells to assess the cytotoxicities of the test compounds (29). In addition, the fluorescence assay described by Kolber et al. (17) and Essig-Marcello and van Buskirk (9) was performed. Briefly, the fluorochrome calcein-acetoxymethyl ester was added to the cultures subsequent to drug exposure for 72 h. After incubation for 1 h, the fluorescent products generated by viable cells were quantified with a CytoFluor (model 2300; Millipore).

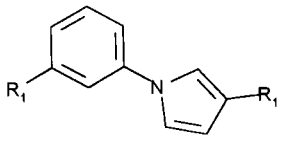
Time versus dose experiment. Experiments to determine the time of exposure to a drug versus the viability (the time-dose response) of *T. brucei rhodesiense* STIB 900 in the presence of the compound CGP 40215A were performed according to a previously published procedure (14). Briefly, trypanosomes were exposed to various drug concentrations for 15 min to 6 days under appropriate culture conditions. After drug exposure, trypanosomes were washed three times and resuspended in fresh drug-free culture medium. For the next 10 days the culture medium was replaced regularly and the trypanosome populations were observed daily. For each exposure time, the lowest drug concentration (MIC) which resulted in irreversible damage over a 10-day period was determined.

Chemicals. CGP compounds were synthesized and made available by the Pharmaceuticals Division, Ciba-Geigy Ltd. (Basel, Switzerland). Suramin (Germanin) was a gift of A. Haberkorn (Bayer AG, Monheim, Germany), and melarsoprol (Arsobal) was a gift of Specia (Paris, France). Diminazene aceturate was obtained commercially.

TABLE 2. Structures and antitrypanosomal activities of biaryl derivatives 21 to 29



21 - 28



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Compound no.	CGP no.	R_1	R_2	R_3	X_1	X_2	X_3	X_4	X_5	X_6	Molecular weight	Formula	*T. brucei rhodesiense* STIB 900	
													MIC (μM)	IC_{50} (μM)
21	39937A	C(=NH)NH_2	H	H	N	CH	CH	N	CH	CH	313.2	$C_{12}H_{12}N_6 \cdot 2HCl$	ND[a]	0.08
22	45631A	C(=NH)N$(CH_3)_2$	H	H	N	CH	CH	N	CH	CH	369.3	$C_{16}H_{20}N_6 \cdot 2HCl$	131	53.6
23	46430A	C(=NH)NH_2	H	H	CH	N	CH	CH	N	CH	313.2	$C_{12}H_{12}N_6 \cdot 2HCl$	143	38.7
24	39711A	C(=NH)NH_2	H	H	CH	CH	N	CH	CH	N	313.2	$C_{12}H_{12}N_6 \cdot 2HCl$	33.5	5.89
25	47185A	C(=NH)NH_2	H	H	CH	CH	CH	CH	CH	CH	347.3	$C_{14}H_{14}N_4 \cdot 2HCl$	1.89	0.31
26	48368A	C(=NH)NH_2	H	CH_3	CH	CH	CH	CH	N	N	327.2	$C_{13}H_{14}N_4 \cdot 2HCl$	34	5.66
27	49233A	C(=NH)NH_2	H	H	CH	CH	CH	N	CH	N	313.2	$C_{12}H_{14}N_6 \cdot 2HCl$	34.3	2.87
28	50073A	C(=NH)NH_2	CH_3	CH_3	N	CH	CH	N	CH	CH	341.3	$C_{14}H_{16}N_6 \cdot 2HCl$	0.14	0.0041
29	42086A	C(=NH)NH_2									300.2	$C_{12}H_{13}N_5 \cdot 2HCl$	3.09	0.16

[a] MIC could not be determined (ND); single trypanosomes survived even at 100 μM.

RESULTS

In a primary screening, 29 AdoMet decarboxylase inhibitors were tested for antitrypanosomal activities against *T. brucei rhodesiense* STIB 900 in vitro. MICs were determined microscopically, and IC_{50}s were determined by a fluorescence assay after a 72-h incubation under axenic conditions (Tables 1 and 2). Nine of the most active compounds were also tested against *T. brucei gambiense* STIB 930, multidrug-resistant *T. brucei brucei* STIB 950, and *T. congolense* STIB 910 (Table 3). The compound most active against *T. brucei rhodesiense*, CGP 40215A, also revealed the highest trypanocidal activities against the other three species tested. For an individual trypanosome species, the rankings of the compounds according to activity were not identical. As an example, CGP 47185A, although the most active compound against *T. brucei gambiense*, had a reduced level of activity against multidrug-resistant *T. brucei brucei* STIB 950. CGP 40215A exhibited levels of activity against the four trypanosome species similar to those of the standard drugs melarsoprol and suramin.

Thirteen of the compounds tested in Basel were examined against a different *T. brucei brucei* isolate (S427) in London (Table 4). In accordance with the results obtained in Basel, eight compounds were found to be inactive (data not presented) while five compounds had IC_{50}s of below 5 μM (Table 4). The superior activities of CGP 40215A and 50073A were also confirmed. Five of the inhibitors examined in Basel were also tested in the Haskins Laboratories in New York against three *T. brucei rhodesiense* isolates (KETRI 243, 269, and 2538) (Table 4). CGP 40215A and 50073A proved to be the most effective inhibitors investigated against these human *T. brucei rhodesiense* isolates.

Five compounds (CGP 40215A, 47185A, 48664A, 50019A, and 50073A) were tested for in vitro activities against *L. do-*

TABLE 3. Activities of selected AdoMet decarboxylase inhibitors and reference drugs for *T. brucei rhodesiense* STIB 900, *T. brucei gambiense* STIB 930, multidrug-resistant *T. brucei brucei* STIB 950, and *T. congolense* STIB 910

Compound no. or drug name	CGP no.	MIC (μM) for:			
		T. brucei rhodesiense	*T. brucei gambiense*	*T. brucei brucei*	*T. congolense*
8	40214A	1.30	10.35	2.69	5.37
10	49776A	2.67	9.15	6.57	4.06
11	48664A	49.2	11	11	ND[a]
12	50019A	2.35	3.31	15.2	3.43
13	52043A	1.40	2.38	4.43	0.53
20	40215A	0.093	1.31	0.18	0.37
25	47185A	1.89	1.19	17.7	1.45
28	50073A	0.14	25.2	11.1	ND
29	42086A	3.09	1.23	15.5	1.23
Diminazene		0.024	0.086	0.215	0.215
Melarsoprol		0.025	0.035	0.084	0.218
Suramin		0.078	0.548	0.233	ND

[a] ND, not done.

TABLE 4. Activities of selected AdoMet decarboxylase inhibitors as determined in three different laboratories with different *T. brucei brucei* subgroup trypanosomes and assays

Compound no.	CGP no.	IC_{50} (μM) determined by listed laboratory for indicated *T. brucei brucei* strain				
		Swiss Tropical Institute[a]	London School[b]	Haskins Laboratories[b]		
		STIB 900	S427	K243	K269	K2538
6	35753A	14.4	48.8	0.45	0.22	11.5
11	48664A	0.49	4.46	1.5	1.85	4.2
12	50019A	0.61	4.45	ND[c]	ND	ND
20	40215A	0.0045	0.78	0.056	0.75	0.11
21	39937A	0.08	ND	0.06	0.02	0.17
25	47185A	0.31	2.34	ND	ND	ND
28	50073A	0.0041	0.29	0.005	0.012	ND

[a] IC_{50}s determined by the fluorescence assay over 3 days.
[b] IC_{50}s based on growth inhibition over 2 days.
[c] ND, not done.

TABLE 5. Toxicities of selected AdoMet decarboxylase inhibitors for human HT-29 cells, and selectivity indices determined with *T. brucei rhodesiense* or *T. brucei gambiense* and HT-29 cells

Compound no.	CGP no.	IC_{50} of HT-29 (mM)	IC_{50} of HT-29/ IC_{50} of *T. b. rhod.*[a]	IC_{50} of HT-29/ IC_{50} of *T. b. gamb.*[b]
8	40214A	1.93	16,600	1,570
10	49776A	2.30	17,700	1,680
11	48664A	15.5	31,600	19,200
12	50019A	4.98	8,200	9,600
13	52043A	9.41	31,400	24,100
20	40215A	1.14	250,000	3,500
21	39937A	2.56	32,000	9,410
25	47185A	2.48	8,000	9,900
28	50073A	1.69	410,000	48,000
29	42086A	1.70	10,600	9,450

[a] *T. b. rhod.*, *T. brucei rhodesiense.*
[b] *T. b. gamb.*, *T. brucei gambiense.*

novani and *T. cruzi*. No inhibition of growth of *L. donovani* could be observed at 90 μM, the highest concentration tested. Against *T. cruzi*, CGP 48664A showed some activity at high concentrations (73% inhibition at 90 μM and 42% inhibition at 30 μM) whereas the other four compounds were not active at 90 μM (data not presented).

The cytotoxicities of selected inhibitors were assessed with the human adenocarcinoma cell line HT-29 (Table 5). For all compounds, the selectivity index (IC_{50} for HT-29 cells/IC_{50} for trypanosomes) was higher than 6,000 for *T. brucei rhodesiense* and higher than 1,570 for *T. brucei gambiense*. Highest indices were calculated for compounds CGP 40215A (250,000) and CGP 50073A (410,000) with *T. brucei rhodesiense*. For *T. brucei gambiense* the indices were in a similar or lower range compared with the indices determined for *T. brucei rhodesiense*.

For the most active compound, CGP 40215A, time-dose responses were investigated. For the concentrations 212 μM (= 100 μg/ml) and 21.2, 2.12, 0.21, and 0.021 μM, the shortest incubation periods which eventually killed the trypanosomes within a 10-day observation period following drug exposure were determined (Fig. 1). For the highest concentration used (212 μM), a 45-min exposure time was required to irreversibly damage the parasites whereas a 30-min incubation time resulted in normal growth over 10 days. With lower concentrations of CGP 40215A, longer exposure times were necessary for irreversible damage. The lowest concentration tested (0.021 μM) did not affect trypanosome viability.

DISCUSSION

In the in vitro evaluation of 29 potential AdoMet decarboxylase inhibitors, 9 demonstrated good levels of activity against *T. brucei rhodesiense* with IC_{50}s of below 0.5 μM. Analysis of the structure-activity relationships revealed that (i) compounds with unmodified termini (amidino- and guanylhydrazone) had higher activity levels than those with modified termini (e.g., CGP 40215A [unmodified] was 2,100-fold more active than CGP 56876A [modified]); (ii) among bicyclic compounds, heterocycles showed lower activity levels than homocycles unless the ring was methylated (e.g., CGP 50073A [heterocyclic methylated] was 20-fold more active than CGP 39937A [heterocyclic]); (iii) all monocyclics had low levels of activity with IC_{50}s of >2 μM; and (iv) compounds with condensed ring systems with unmodified amidino- and guanylhydrazone termini were active; e.g., substitution of an amino group by an oxygen decreased activity significantly (i.e., CGP 48664A [unmodified] was 167-fold more active than CGP 53392A [modified guanylhydrazone terminus]). CGP 40215A appears to be the optimal structure among the compounds tested, combining most of the favorable structural features improving antitrypanosomal activity. CGP 40215A is bicyclic and homocyclic and contains unmodified termini. In addition, CGP 50073A is another highly active compound which also fulfills the criteria mentioned above.

The calculated MICs in Tables 1, 2, and 3 represent those concentrations of agents needed to completely inhibit growth, while the IC_{50}s represent 50% inhibition of growth. The MIC/IC_{50} ratio may thus be important, since low values may be an indication of potential in vivo efficacy. In examining the ratios calculated from data from Tables 1, 2, and 3 (not shown), 50% are between 2 and 5, with most of the remainder in the 6 to 25 range. Interestingly, the two compounds having the lowest

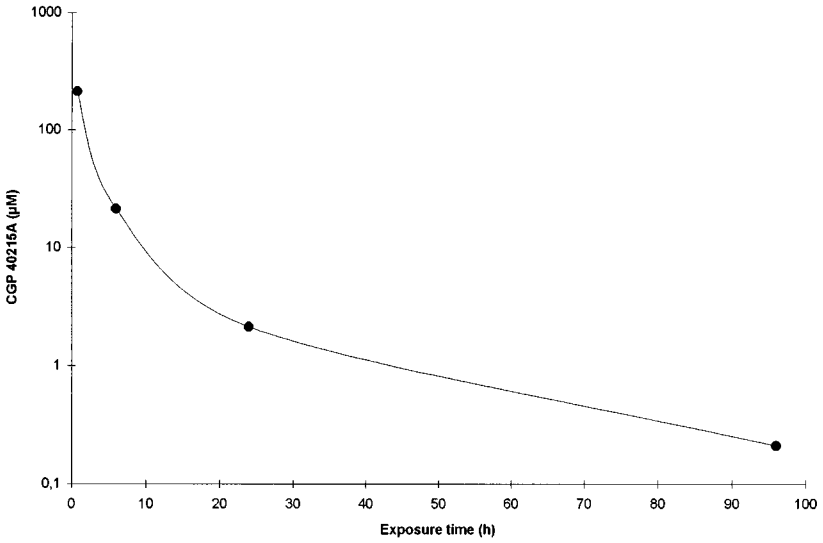

FIG. 1. Time-dose responses of *T. brucei rhodesiense* STIB 900 in the presence of CGP 40215A. The curve represents the minimum dose exposure time required to irreversibly damage the trypanosomes. Any time-dose combination above the curve is sufficient to eliminate the parasites.

IC_{50}s, CGP 40215A and 50073A, had MIC/IC_{50} ratios which were among the highest (21 and 34, respectively). Since these agents were also most active in in vivo studies (1), it does not appear that the MIC/IC_{50} ratio is significant for the evaluation of this series of compounds.

Seven compounds were examined in three different laboratories, each one having its own assay and a different *T. brucei* sp. population. CGP 40215A and 50073A were the most active compounds in the ranking of each laboratory. The lower IC_{50}s obtained at the Swiss Tropical Institute, Basel, compared with those of the other two laboratories can be explained by the longer drug exposure period (3 days compared with 2 days). Interestingly, compounds CGP 39937A and 40215A proved to be effective against arsenic-resistant K243 and diamidine-resistant K269 (4).

The promising antitrypanosomal activity of CGP 40215A was confirmed for other trypanosome species (*T. brucei gambiense* and *T. congolense*), including multidrug-resistant *T. brucei brucei*. MICs of CGP 40215A were in a range similar to those of the commercial trypanocides melarsoprol, suramin, and diminazene.

All active compounds expressed a selective in vitro antitrypanosomal activity which was not due to a general cytotoxicity. The calculated selectivity index (HT-29/*T. brucei rhodesiense*) was highest for CGP 40215A and 50073. However, the index of 250,000 for CGP 40215A cannot simply be transferred to the in vivo situation. Toxic effects were observed during prolonged (14-day) intraperitoneal administration of 50 mg/kg of body weight in mice (1). Nevertheless, selectivity indices of the compounds investigated were in a range similar to or higher than those of suramin and melarsoprol (unpublished data).

The time-dose experiment with *T. brucei rhodesiense* and CGP 40215A demonstrated that the effect of this molecule is time and dose dependent. An exposure for 6 days to 0.021 μM had no adverse effect on the trypanosomes nor did a 3-h exposure to 21.1 μM. This has implications for in vivo experiments in that a trypanocidal concentration has to be maintained for a certain period of time, especially in the cerebrospinal fluid in a case of central nervous system infection. A similar experiment has been carried out with diminazene aceturate and a *T. brucei brucei* isolate (14). Much shorter exposure times were required for diminazene at comparable concentrations to exert a trypanocidal effect.

The fact that a transient exposure of trypanosomes to the AdoMet decarboxylase inhibitor CGP 40215A (e.g., 45 min at 100 μg/ml [212 μM]) caused persistent effects lasting for at least 10 days may indicate that either the compound was retained inside the organisms or effects independent of AdoMet decarboxylase inhibition and polyamine depletion were exerted by high drug concentrations. Preliminary experiments indicated that CGP 40215A is a competitive inhibitor of trypanosomal AdoMet decarboxylase (data not presented). Additional studies presented in a related report indicate that trypanosomes incubated with CGP 40215A for 1 h have reduced levels of AdoMet decarboxylase activity and reduced levels of synthesis of spermidine (1). Experiments to quantitate the drug concentration in trypanosomes at various times after a short drug exposure are under way.

The excellent in vitro activities of some of the tested compounds, especially CGP 40215A and 50073A, and the low levels of in vitro cytotoxicity encourage further evaluation of these compounds in rodent models for trypanosomiasis (1).

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