ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2009, p. 3269–3272 Vol. 53, No. 8
0066-4804/09/$08.00+0 doi:10.1128/AAC.00076-09

Trypanocidal Activity of 8-Methyl-5′-{[(*Z*)-4-Aminobut-2-enyl]-(Methylamino)}Adenosine (Genz-644131), an Adenosylmethionine Decarboxylase Inhibitor▽

Cyrus J. Bacchi,[1,2]* Robert H. Barker, Jr.,[1,3] Aixa Rodriguez,[1] Bradford Hirth,[3] Donna Rattendi,[1] Nigel Yarlett,[1,4] Clifford L. Hendrick,[3] and Edmund Sybertz[3]

Haskins Laboratory[1] and Department of Biological and Health Sciences, Pace University,[2] New York, New York 10038; Genzyme Corp., Waltham, Massachusetts 02451[3]; and Department of Chemistry and Physical Sciences, Pace University, New York, New York 10038[4]

Received 20 January 2009/Returned for modification 6 April 2009/Accepted 11 May 2009

Genzyme 644131, 8-methyl-5′-{[(*Z*)-4-aminobut-2-enyl](methylamino)}adenosine, is an analog of the enzyme activated *S*-adenosylmethionine decarboxylase (AdoMetDC) inhibitor and the trypanocidal agent MDL-7381, 5-{[(*Z*)-4-aminobut-2-enyl](methylamino)}adenosine. The analog differs from the parent in having an 8-methyl group on the purine ring that bestows favorable pharmacokinetic, biochemical, and trypanocidal activities. The compound was curative in acute *Trypanosoma brucei brucei* and drug-resistant *Trypanosoma brucei rhodesiense* model infections, with single-dose activity in the 1- to 5-mg/kg/day daily dose range for 4 days against *T. brucei brucei* and 25- to 50-mg/kg twice-daily dosing against *T. brucei rhodesiense* infections. The compound was not curative in the TREU 667 central nervous system model infection but cleared blood parasitemia and extended time to recrudescence in several groups. This study shows that AdoMetDC remains an attractive chemotherapeutic target in African trypanosomes and that chemical changes in AdoMetDC inhibitors can produce more favorable drug characteristics than the lead compound.

Sleeping sickness or human African trypanosomiasis infects between 50,000 and 150,000 people each year across sub-Saharan Africa and is fatal if left untreated. Yearly estimates of people at risk are 10 million on the African continent. Current drugs for late stage disease, such as melarsoprol, have significant toxicity and resistance to melarsoprol is increasing. Another drug, eflornithine, requires 2 weeks of intravenous infusion, which is highly impractical in rural Africa (10). A promising new combination regimen for late-stage disease that appears to be effective uses eflornithine for 1 week plus oral nifurtimox for 10 days (15). This is a small-scale trial that needs to be reinforced with more data. Nevertheless, new therapies are urgently needed; because of the extreme poverty in countries with endemic disease, there has been little interest for many years within the pharmaceutical industry in discovering and developing new drugs to treat a disease that occurs primarily in developing countries (10).

Polyamine metabolism of African trypanosomes has been shown to be a valid chemotherapeutic target for inhibitors aimed at critical points in the pathway such as ornithine decarboxylase (2), trypanothione synthase (11, 13), and *S*-adenosylmethionine decarboxylase (AdoMetDC) (6). AdoMetDC has been shown to be an essential enzyme in the trypanosome polyamine pathway, both by RNA interference knockdown (18, 19) and through the use of inhibitors, such as MDL-73811 (8), which kill parasites both in vitro (1) and in vivo (4, 6). However, while potent, MDL-73811 lacks the pharmacokinetic and tissue (central nervous system [CNS]) distribution characteristics that are essential to meet the improved target product profile for a new late-stage antitrypanosomal drug (7). We therefore started a program to synthesize analogs with high potency and better pharmacodynamic properties. Genz-644131, the 8-methyl analog of MDL-73811, was significantly more potent in vivo against trypanosomes than other analogs made at that time (5). Some adenine C-8-substituted analogs were recently tested as inhibitors of human AdoMet decarboxylase but not against the trypanosome enzyme or versus trypanosomes in vitro. (14).

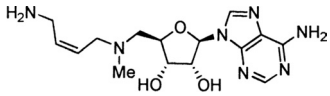
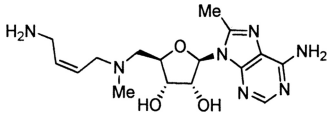
MATERIALS AND METHODS

Chemical synthesis. MDL-73811 was synthesized as a benchmark using methods that have been previously described (15). The synthesis of Genz-644131 is described elsewhere in detail (see supplemental material for reference 17) and briefly outlined here. 8-Bromoadenosine was O protected with hexamethyldisilazane and subjected to palladium-catalyzed coupling with trimethylaluminum to install the 8-methyl group. The product was then deprotected and treated with thionyl chloride in pyridine to afford the 5′-chloro derivative. This intermediate was converted to the 5′-methylamino compound by treatment with excess methylamine and then alkylated with *cis*-1-(*t*-butoxycarbonylamino)-4-chloro-2-butene to furnish the protected product. The synthesis of Genz-644131 was completed by acid catalyzed cleavage of the terminal *N*-butoxylcarbonyl group using methanolic hydrogen chloride.

In vitro trypanosome assays. In vitro trypanosome 50% inhibitory concentration (IC_{50}) growth assays were performed as previously described (16) using *Trypanosoma brucei brucei* Lab 110 EATRO and *Trypanosoma brucei rhodesiense* strains KETRI 243 and 2538 (3). Drug studies were done in duplicate in 24-well plates (1 ml/well) with final inhibitor concentrations of 0.1, 1.0, 10, and 100 μM. After 48 h. the parasites were counted in a Z-1 Coulter Counter, and the approximate range of activity was determined. The IC_{50}s were then determined from additional studies using closely spaced inhibitor concentrations. Analogs were dissolved in water, and dilutions were made with HMI-18 medium. The results are reported as the averages from two experiments.

* Corresponding author. Mailing address: Haskins Laboratories, Pace University, 41 Park Row, New York, NY 10038. Phone: (212) 346-1246. Fax: (212) 346-1586. E-mail: cbacchi@pace.edu.
▽ Published ahead of print on 18 May 2009.

TABLE 1. In vitro activity against trypanosome strains

Compound	Structure	IC_{50} (μg/ml)[a]		
		T. brucei brucei Lab 110 EATRO	*T. brucei rhodesiense* KETRI 243	*T. brucei rhodesiense* KETRI 2538
MDL-73811		0.05	0.02	0.021
Genz-644131		0.00058	0.00375	0.0003

[a] The fold increases in activity for Genz-644131 were 86.2, 5.3, and 70 for *T. brucei brucei* Lab 110 EATRO, *T. brucei rhodesiense* KETRI 243, and *T. brucei rhodesiense* KETRI 2538, respectively.

In vivo trypanosome assays. In vivo studies were performed examining efficacy of Genz-644131 against acute model infections: *T. brucei brucei* (Lab 110 EATRO strain) and *T. brucei rhodesiense* (KETRI 243, 1992, and 2002 strains) as previously described (4). Briefly, groups of five animals were infected intraperitoneally (i.p.) on day 0 with 2.5×10^5 parasites, and dosing was initiated on Day 1. Genz-644131 was dosed at a 1- to 50-mg/kg/day regimen i.p. either once a day (QD) or twice a day (BID) for 4 days. Animals were assessed twice weekly by microscopic examination of at least 20 fields of wet blood smears. Animals surviving >30 days beyond death of the last untreated control with no evidence of parasites in tail vein blood were considered cured. In this model, untreated animals generally were moribund and were euthanized by days 3 to 4. Treatment with pentamidine at 2 mg/kg QD for 4 days served as a positive control in all acute model infections.

CNS model infections. The TREU 667 model CNS infection developed by Jennings et al. (12) was used to evaluate Genz-644131 versus CNS disease. In this model, mice were infected with 10,000 trypanosomes from an initial rat transfer, and the infection was allowed to develop for 21 days, at which time there is CNS involvement. Berenil (10 mg/kg i.p. [once]; diminazene aceturate) will initially clear the blood parasites at day 21, but since it does not cross the blood-brain barrier, the blood will eventually be repopulated from the CNS as reservoir. A day 4 Berenil-treated group (10 mg/kg i.p. [once]) served as a positive control. At day 21, mice with confirmed parasitemia were randomly separated into groups of 10, and treatment was begun. Mice were checked weekly for parasitemia, starting 7 days after the final dosing. Animals recrudescing with parasites in tail vein blood samples (magnification, ×400; 20 fields) were euthanized. The animals were monitored for 6 months after the last dosing. The animals surviving this period were euthanized; their brains were homogenized, and samples were injected into two healthy animals (9).

RESULTS

MDL-73811 and Genz-644131 are highly active against *T. brucei brucei* in vitro. The IC_{50} of MDL-73811 for *T. brucei brucei* Lab 110 EATRO was 0.05 μg/ml (Table 1). In contrast, Genz-644131 was ~100-fold more potent versus this isolate (0.00058 μg/ml [0.0096 μM] versus 0.05 μg/ml [0.083 μM]). The IC_{50}s for the two *T. brucei rhodesiense* isolates were correspondingly lower with Genz-644131 than with MDL-73811. Recent studies (5) showed that Genz-644131 was also a more potent inhibitor of purified AdoMetDC heterodimeric enzyme than MDL-73811 ($k_{inact}/Ki^{app} = 7.8\ M^{-1}\ min^{-1}$ versus $k_{inact}/Ki^{app} = 1.5\ \mu M^{-1}\ min^{-1}$).

Genz-644131 is active against an acute murine model of trypanosome infection. In an initial in vivo study (5), we found Genz-644131 at 50 mg/kg/day QD or BID cured animals with a 24-h infection of the *T. brucei brucei* Lab 110 EATRO model. These results were equivalent to those obtained with the parent compound, MDL-73811. Genz-644131 was then studied in a series of experiments with the Lab 110 EATRO model using lower-dose regimens. These experiments (the results are presented in Table 2) indicate highly curative activity at 1, 2, 2.5, and 5 mg/kg/day, both QD and BID. The only dose level at which >50% cures were not obtained was 1 mg/kg QD for 4 days. However, even at this dose, a 33% cure was obtained, with the remaining animals having almost three times the survival time of untreated controls (Table 2).

Activity versus *T. brucei rhodesiense* clinical isolates. Genz-644131 was tested for activity versus three Kenya Trypanosomiasis Research Institute *T. brucei rhodesiense* strains: KETRI 2002, pentamidine and melarsoprol sensitive; KETRI 1992, pentamidine resistant and melarsoprol sensitive; and KETRI 243, pentamidine and melarsoprol resistant (Table 3). All dosing was QD or BID for 4 days. Genz-644131 was effective against KETRI 2002, curing 60% of the animals at 10 mg/kg BID and 80% at 50 mg/kg/day QD or BID, and against KETRI

TABLE 2. Susceptibility of *T. brucei brucei* Lab 110 EATRO to Genz-644131 and pentamidine

Compound[a]	Dose (mg/kg)	Frequency[b] (i.p.)	Time (days)	Mean survival time (days)[c]	No. of mice cured/ total no. of mice tested[d]
Control				4.7	0/21*
Pentamidine	2	QD	4	>35	21/21*
Genz-644131	1	QD	4	13.5	1/3
	2	BID	4	>35	3/3
	2	QD	4	>35	3/3
	2.5	QD	4	21	2/3
	2.5	BID	4	>35	3/3
	5	QD	4	>35	3/3
	5	BID	4	>35	3/3
	10	QD	4	>35	3/3
	10	BID	4	>35	8/8
	25	QD	4	>35	5/5
	25	BID	4	>35	5/5
	50	QD	4	>35	5/5
	50	BID	4	>35	5/5

[a] Mice were infected with 2.5×10^5 trypanosomes, and treatment was begun 24 h later. Control mice were not treated.
[b] BID dosing occurred at approximately 10 a.m. and 4 p.m.
[c] That is, the mean survival time of animals dying of trypanosomiasis, exclusive of cured animals.
[d] Cured animals survived >30 days beyond the deaths of the untreated controls, with no parasites in the tail vein blood smears. *, Composite of five experiments.

TABLE 3. Susceptibility of *T. brucei rhodesiense* KETRI strains to Genz-644131[a]

Compound (dosing frequency)[b]	Dose (mg/kg)	No. of mice cured/total no. of mice tested (avg survival time [days])[c]		
		KETRI 2002	KETRI 1992	KETRI 243
Control		0/5 (10.2)	0/5 (11.2)	0/5 (10.6)
Pentamidine (QD)	2	5/5 (−)	0/5 (18)	0/5 (11.6)
Genz- 644131 (QD)	10	0/5 (16)	0/5 (16.6)	0/5 (13.2)
	25	1/5 (20)	0/5 (18.6)	0/5 (14)
	50	4/5 (21)	0/5 (20.2)	0/5 (17.2)
Genz-644131 (BID)	10	3/5 (21.5)	0/5 (20.4)	0/5 (14.8)
	25	2/5 (19.3)	3/5 (23)	1/5 (18.75)
	50	4/5 (22)	5/5 (−)	2/5 (23.3)

[a] Mice were infected with 2.5×10^5 trypanosomes from rats infected with frozen stabilates. Dosing was begun i.p. at 24 h after infection. All dosing was for 4 days.
[b] BID dosing occurred at approximately 10 a.m. and 4 p.m.
[c] Exclusive of cured animals. Cured animals survived >30 days beyond the deaths of untreated controls, with no parasites in the tail vein blood smears.

TABLE 4. Genz-644131 versus *T. brucei brucei* 667

Compound and group	Dose (mg), time (days)	No. of mice dead prior to dosing completion	No. of mice relapsing	Avg day of relapse (range)	No. of mice remaining in group
None					
1		2	8		0
Berenil					
2	10, 4	0	1	36 (36)	9
3	10, 21	0	10	61.8 (43–77)	0
644131[a]					
4	10, 7	2	7	53 (43–72)	1
5	25, 7	0	10	61.7 (43–92)	0
6	50, 7	3	7	59 (57–64)	0
7	100, 7	1	9	68.8 (64–78)	0
8	10, 14	2	2	59.8 (43–72)	0
9	25, 14	2	8	70.3 (57–92)	0
10	50, 14	0	9[b]	63.4 (50–72)	0
11	100, 14	5	5	72 (64–78)	0

[a] All dosing was BID.
[b] One animal in the group was euthanized on day 44. It was not trypanosome positive but was ill for unknown reasons.

1992, curing 60% at 25 mg/kg/day BID and 100% at 50 mg/kg/day BID. At 50 mg/kg, Genz-644131 cured two of five of the KETRI 243-infected animals. Lower doses resulted in prolonged survival times for animals infected with this difficult-to-cure strain. Note that pentamidine did not cure KETRI 1992- or KETRI 243-infected animals.

CNS model infection. The TREU 667 model has been used for many years as a reliable indicator of laboratory CNS disease, and the potential of agents curing this model are viewed as having potential for curing stage 2 clinical disease in humans. In the present study, we used Genz-644131 at 10, 25, 50, and 100 mg/kg/day given i.p. BID for 1 or 2 weeks (Table 4). Unfortunately, all of the animals, except one at 10 mg/kg for 7 days, eventually relapsed. Genz-644131 did initially clear the blood of parasites and increased the time to relapse in several dose groups beyond that of the Berenil day 21 control (61.8 days), and appeared nontoxic at higher doses for extended periods.

Although treatment with Genz-644131 did not ultimately prevent relapse, it did significantly increase time to relapse compared to untreated controls ($P < 0.0001$ for both 7- and 14-day treatment as determined by a Mantel-Cox log-rank test). However, even at 100 mg/kg for 14 days, the relapse time for Genz-644131 was not significantly different from the relapse achieved with Berenil treatment on day 21 ($P < 0.0964$).

DISCUSSION

The compound MDL-73811 was the initial lead in Merrell-Dow's development of AdoMetDC inhibitors as antiparasitic agents in the late 1980s. MDL-73811 was an enzyme-activated inhibitor reminiscent of the ornithine decarboxylase agent DL-α-difluoromethylornithine. In a series of studies (4, 6, 7), MDL-73811 was found to cure *T. brucei brucei* and *T. brucei rhodesiense* murine laboratory model infections. This compound was ~100-fold more active in this regard than DFMO (6), curing at 20 to 50 mg/kg/day for 5 days. Despite excellent activity versus acute model infections, MDL-738911, used alone, was incapable of curing a model CNS infection (4).

Genz-644131 showed superior properties to MDL-73811: a 5- to 85-fold greater potency in vitro (Table 1), a 5-fold higher inhibitory activity against purified AdoMetDC enzyme, an ~3-fold longer blood half-life, an 8-fold higher maximum concentration in brain, and only 41% serum protein binding (5). These studies, along with associated pharmacokinetic values, suggested that plasma levels above IC_{50}s could be achieved over a 24-h period with a single dose of 5 mg/kg. This prediction was validated in acute infections in which single daily doses of 1, 2, 2.5, and 5 mg/kg produced cures of *T. brucei brucei* Lab 110 EATRO (Table 2). This is considerably below the 10 to 20 mg/kg 3 x/day needed to cure with MDL-73811 (6). In the present study 2 of 3 strains of *T. brucei rhodesiense* were successfully treated with i.p. dosing at 10 to 50 mg/kg/day BID dosing (Table 3), whereas constant infusion of MDL-73811 by osmotic pump (50 mg/kg for 7 days or at 50 mg/kg three times daily for 5 days) was needed to cure a *T. brucei rhodesiense* strain (4). Thus, the improvement in pharmacokinetic properties has clearly led to increased efficacy in acute infections. Although Genz-644131 did not cure a CNS infection at up to 100 mg/kg BID for 14 days, its activity versus acute infections and *T. brucei rhodesiense*, along with favorable biochemical and pharmacokinetic properties, indicate that this chemical series, with further adjustment of brain penetration capabilities, has significant potential for the development of effective nontoxic trypanocides for late-stage disease. Preceding work with CGP-40215, an AdoMetDC inhibitor that cured 15 laboratory infections with clinical isolates, was also not curative with the TREU 667 model when used singly at 10 to 50 mg/kg/day for 14 days (1). That report plus the present study and studies on MDL-73811 do, however, validate AdoMetDC in trypanosomes as a drug target and encourage additional pharmacokinetic studies to maximize blood-brain penetration.

ACKNOWLEDGMENTS

We thank Patrick Casara, Simon Croft, Reto Brun, and Robert Don for helpful discussions throughout these studies. We also thank Allison Kosciolek and Ali Hussain for technical assistance.

This study was supported in part by funding from DNDi and by resources provided by Genzyme.

REFERENCES

1. **Bacchi, C. J., R. Brun, S. L. Croft, K. Alicea, and Y. Buhler.** 1996. In vivo trypanocidal activities of new *S*-adenosylmethionine decarboxylase inhibitors. Antimicrob. Agents Chemother. **40:**1448–1453.
2. **Bacchi, C. J., H. C. Nathan, S. H. Hutner, P. P. McCann, and A. Sjoerdsma.** 1980. Polyamine metabolism: a potential therapeutic target in trypanosomes. Science **210:**332–334.
3. **Bacchi, C. J., H. C. Nathan, T. Livingston, G. Valladares, M. Saric, P. D. Sayer, A. R. Njogu, and A. B. Clarkson, Jr.** 1990. Differential susceptibility to DL-α-difluoromethylornithine in clinical isolates of *Trypanosoma brucei rhodesiense*. Antimicrob. Agents Chemother. **34:**1183–1188.
4. **Bacchi, C. J., H. C. Nathan, N. Yarlett, B. Goldberg, P. P. McCann, A. J. Bitonti, and A. Sjoerdsma.** 1992. Cure of murine *Trypanosoma brucei rhodesiense* infections with an *S*-adenosylmethionine decarboxylase inhibitor. Antimicrob. Agents Chemother. **36:**2736–2740.
5. **Barker, R. H., Jr., H. Liu, B. Hirth, C. A. Celatka, R. Fitzpatrick, Y. Xiang, E. K. Willert, M. A. Phillips, M. Kaiser, C. J. Bacchi, A. Rodriguez, N. Yarlett, J. D. Klinger, and E. Sybertz.** 2009. Novel *S*-adenosine decarboxylase inhibitors for the treatment of human African trypanosomiasis. Antimicrob. Agents Chemother. **53:**2052–2058.
6. **Bitonti, A. J., T. L. Byers, T. L. Bush, P. J. Casara, C. J. Bacchi, A. B. Clarkson, Jr., P. P. McCann, and A. Sjoerdsma.** 1990. Cure of *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense* infections in mice with an irreversible inhibitor of *S*-adenosylmethionine decarboxylase. Antimicrob. Agents Chemother. **34:**1485–1490.
7. **Byers, T. L., T. L. Bush, P. P. McCann, and A. J. Bitonti.** 1991. Antitrypanosomal effects of polyamine biosynthesis inhibitors correlate with increases in *Trypanosoma brucei brucei S*-adenosyl-L-methionine. Biochem. J. **274**(Pt. 2)**:** 527–533.
8. **Casara, P., P. Marchal, J. Wagner, and C. Danzin.** 1989. 5′-{Smethylamino}-5′-deoxyadenosine: a potent enzyme-activated irreversible inhibitor of *S*-adenosyl-L-methionine decarboxylase from *Escherichia coli*. J. Am. Chem. Soc. **111:**9111–9113.
9. **Clarkson, A. B., Jr., E. J. Bienen, C. J. Bacchi, P. P. McCann, H. C. Nathan, S. H. Hutner, and A. Sjoerdsma.** 1984. New drug combination for experimental late-stage African trypanosomiasis: DL-α-difluoromethylornithine (DFMO) with suramin. Am. J. Trop. Med. Hyg. **33:**1073–1077.
10. **Delespaux, V., and H. P. de Koning.** 2007. Drugs and drug resistance in African trypanosomiasis. Drug Resist. Update **10:**30–50.
11. **Fairlamb, A. H., G. B. Henderson, C. J. Bacchi, and A. Cerami.** 1987. In vivo effects of difluoromethylornithine on trypanothione and polyamine levels in bloodstream forms of *Trypanosoma brucei*. Mol. Biochem. Parasitol. **24:**185–191.
12. **Jennings, F. W., D. D. Whitelaw, and G. M. Urquhart.** 1977. The relationship between duration of infection with Trypanosoma brucei in mice and the efficacy of chemotherapy. Parasitology **75:**143–153.
13. **Krauth-Siegel, L. R., M. A. Comini, and T. Schlecker.** 2007. The trypanothione system. Subcell. Biochem. **44:**231–251.
14. **McCloskey, D. E., S. Bale, J. A. Secrest, A. Tiwari, T. H. Moss, J. Valiyaveettil, W. H. Brooks, W. C. Guida, A. E. Pegg, and S. E. Ealick.** 2009. New insights into the design of inhibitors of human *S*-adenosylmethionine decarboxylase: studies of adenine C(8) substitution in structural and analogues of *S*-adenosylmethione. J. Med. Chem. [Epub ahead of print.]
15. **Priotto, G., S. Kasparian, D. Ngouama, S. Ghorashian, U. Arnold, S. Ghabri, and U. Karunakara.** 2007. Nifurtimox-eflornithine combination therapy for second-stage *Trypanosoma brucei gambiense* sleeping sickness: a randomized clinical trial in Congo. Clin. Infect. Dis. **45:**1435–1442.
16. **Sufrin, J. R., D. Rattendi, A. J. Spiess, S. Lane, C. J. Marasco, Jr., and C. J. Bacchi.** 1996. Antitrypanosomal activity of purine nucleosides can be enhanced by their conversion to O-acetylated derivatives. Antimicrob. Agents Chemother. **40:**2567–2572.
17. **Willert, E. K., and M. A. Phillips.** 2007. Allosteric regulation of an essential trypanosome polyamine biosynthetic enzyme by a catalytically dead homolog. Proc. Natl. Acad. Sci. USA **104:**8275–8280.
18. **Willert, E. K., and M. A. Phillips.** 2006. Genetic knockdown of *S*-adenosylmethionine decarboxylase. Fourth Biannual Polyamines in Parasites Conference, Portland, OR.
19. **Willert, E. K., and M. A. Phillips.** 2008. Regulated expression of an essential allosteric activator of polyamine biosynthesis in African trypanosomes. PLoS Pathog. **4:**e1000183.