1-(Substituted)benzyl-5-aminoimidazole-4-carboxamides are potent orally active inhibitors of *Trypanosoma cruzi* in mice

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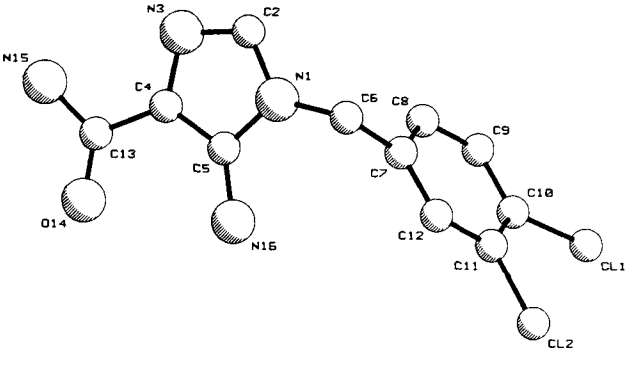
Summary. 1-(Substituted)benzyl-5-aminoimidazole-4-carboxamides are potent orally active inhibitors of *Trypanosoma cruzi* infections in mice. The most active compounds are the 1-(4-chlorobenzyl)- and 1-(3,4-dichlorobenzyl)-analogs (L-153,094 [**2**] and L-153,153 [**4**], resp.) which are approximately 7-fold more potent upon oral administration than nifurtimox (Lampit) in suppressing parasite levels in the blood of mice with acute *Trypanosoma cruzi* infections.
Key words. 1-(Substituted)-benzyl-5-aminoimidazole-4-carboxamide; *T. cruzi*; Chagas' disease.

An estimated 16–18 million people in Central and South America suffer from Chagas' disease, a debilitating and frequently fatal chronic parasitic infection caused by the protozoan parasite *Trypanosoma cruzi*. Approximately 20–30% of infected individuals develop chagasic cardiopathy [1] and in Brazil, Chagas' disease was responsible for 10% of all adult deaths in 1982 [2]. No drug has been found that is able to prevent or cure Chagas' disease. The two most widely used drugs, nifurtimox (Lampit) [3] and benznidazole (Rochagan, Radinil) [4], are only partially effective in treating the acute disease and are ineffective in the chronic disease. Strains resistant to these drugs have also appeared. Because of the relative lack of potency of nifurtimox and benznidazole and the consequent long-term high dosage regimen required, serious side effects such as anorexia, skin rash, peripheral neuritis, and psychosis often occur [5]. The critical need for a better agent for the treatment of Chagas' disease has been recognized for some time.

We wish to report the discovery that several 1-(substituted)benzyl-5-aminoimidazole-4-carboxamides suppress circulating parasite levels in mice with acute infections of *T. cruzi*, and that among the most potent of these analogs, 1-(3,4-dichlorobenzyl)-5-aminoimidazole-4-carboxamide (L-153,153, **4**), is approximately 7.5 times more potent than nifurtimox in this model (SD_{50} 1.75 mg/kg/day for 5 days, compared to 13.2 mg/kg/day for Lampit). Similarly, 1-(4-chlorobenzyl)-5-aminoimidazole-4-carboxamide (L-153,094, **2**) also exhibited greater potency than nifurtimox, with an SD_{99} about 7 times lower. Neither analog exhibited gross signs of toxicity when dosed at 200 mg/kg/day, i.e. > 100 times SD_{50} values.

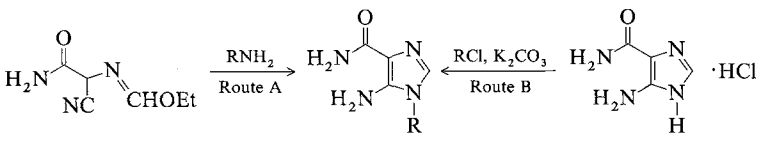
Chemistry. 1-(Substituted)-5-aminoimidazole-4-carboxamides **1–7** were prepared by two general procedures (table 1). Route A, which is a modification of a procedure introduced by Robinson and Shaw [6], involved the in situ generation of formimidate **I** from 2,2-aminocyanoacetamide and triethyl orthoformate in acetonitrile and its subsequent reaction at reflux with a primary amine (RNH_2) to give the desired imidazole product. Alternatively, in route B 5-aminoimidazole-4-carboxamide **II** [7] was alkylated with a 10–50% excess of alkyl halide (RX) in hot acetone in the presence of powered K_2CO_3. The products generally precipitated from the reaction mixture upon partial concentration or on dilution with water. Structural assignment in this alkylation reaction was based on comparison of compound **1** prepared by both routes. Additional confirmation of the site of alkylation in L-153,153 (**4**) was obtained by the observation of a nuclear Overhauser effect of 4% on the 5-amine protons upon irradiation of the benzylic methylene protons, and by X-ray crystallographic analysis (fig.).

Biology. The experimental protocol used for the in vivo testing of chemical analogs is essentially the same as that described earlier [8]. Female CF_1 albino mice (4–6 weeks old, Carworth Farms, Portage, Michigan, USA) and an unclassified Brazilian strain of *Trypanosoma cruzi*, which has been maintained in this laboratory for several years, were used for the primary screening experiments. Each test mouse was infected by means of the intraperitoneal injection of 50,000 trypomastigotes (obtained from the blood of heavily infected donor mice) and the infection was allowed to progress for 10 days. Test compounds were orally administered to each of six mice twice daily in 0.1% Tween 80 plus 0.5% hydroxyethylcellulose vehi-



A computer-generated drawing of L-153,153 (**4**) as determined by X-ray crystallography confirming the site of alkylation at N_1. Hydrogen atoms are omitted for clarity.

Table 1. Preparation and biological activity of selected 5-aminoimidazole-4-carboxamides



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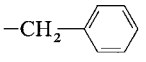
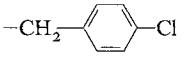
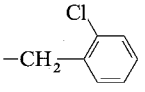
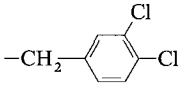
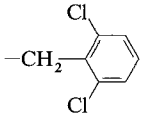
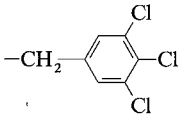
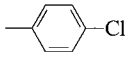
Compound	R	Route	MP (°C)	% Yield	Dose (mg/kg/day)	% Suppression
1		A,B	255-9	65, 47	200	12
2	 L-153,094	B	276-8	62	50 25 12.5 6.25 3.13	100 98.5 96.5 93 81
3		B	231-4	70	200	3
4	 L-153,153	B	247-9	60	200 100 50 25 12.5	100 99 97.5 95 93
5		B	251.6	86	200	16
6		B	286-7.5	38	200	45
7		A	262-3	35	200	9

cle on days 11 through 14 at a dosage level of 200 mg/kg/day. Nifurtimox was administered at varying dosage levels to groups of six mice to serve as positive control. One group of six mice received the vehicle only to serve as negative controls. Blood smears were prepared on day 15 postinfection and the numbers of parasites per milliliter of blood determined as previously described[9]. Group total body weights were obtained on all test mice immediately prior to initiation of administration of test compounds and on day 15 (1 day after completion of treatment). Body weight changes were used as a general indication of the toxicity of the test compounds, and mice were observed for other gross signs of toxicity such as roughened hair coat and abnormal behavior.

Additional studies on selected promising drugs were carried out at decreasing dose levels until an end point of activity was reached. The values obtained for test compounds were plotted along with those obtained for the reference compound, nifurtimox, on log-dose/probit-activity graph paper from which doses producing 50% parasite suppression (SD_{50}) can be interpolated.

Because of the high susceptibility of C_3H inbred female mice to this strain of *T. cruzi*[10], these hosts were used to compare the curative efficacy of L-153,094 with that of nifurtimox. Following injection of C_3H mice by the intraperitoneal injection of each with 50,000 trypomastigotes of *T. cruzi*, treatment with nifurtimox (100 mg/kg/day) and L-153,094 (50 mg/kg/day) was initiated on day 11 post infection and continued through day 22. Blood smears were made on days 25 and 46 following infection and examined microscopically for the presence of parasites. Those mice found positive were euthanized. Any

Table 2. Summary of the suppressive activity of L-153,153 and Lampit against *Trypanosoma cruzi* in the CF_1 mouse

Representative experiment	Treatment	mg/kg/day	Percent suppression[1]
1	Lampit	100	98.7
		50	85.0
	L-153,153	200	100.0
2	Lampit	100	99.1
		25	84.4
	L-153,153	200	98.7
		100	99.0
		50	98.9
3	Lampit	100	97.9
		25	72.2
	L-153,153	50	96.3
		25	95.4
		12.5	93.3
4	Lampit	100	100.0
		50	93.0
		25	88.0
	L-153,153	12	92.6
		6	82.1
		3	72.0
5	Lampit	100	99.0
		50	96.0
		25	84.0
	L-153,153	3	66.4
		1	32.6

[1] The mean number of parasites/ml of blood in the vehicle treated group compared with the mean number of parasites/ml of blood in the drug treated group[9].

Table 3. Comparison of the curative effects of L-153,094 and Lampit against patent infections of *Trypanosoma cruzi* in C_3H mice

Treatment	mg/kg/day	Deaths Day 25	Microscopic results Day 25	Day 46	Day 70
Vehicle control	–	6/7	+ (1)	ND	ND
Lampit	100	0/7	– (7)	+ (7)	ND
L-153,094	50	0/7	+ (3) – (4)	+ (5) – (2)	+ (5)[a] – (3)[a]

ND: Not done. All mice positive or dead. + and – refer to presence and absence of parasites, respectively, and numbers in parentheses are numbers of mice. [a] Results from subinoculation into uninfected C_3H mice.

mice found negative were bled on day 48 and the blood from each subsequently subinoculated into 4 uninfected C_3H mice. The recipient mice were then checked for the presence of parasites by the microscopic examination of fresh blood taken 22 days after subinoculation.

Results and discussion

Although structure-activity relationships for activity against *T. cruzi* in this model were not explored extensively, several points are clear from inspection of table 1. Comparison of **7** with **2** demonstrates that the benzyl methylene is critical to activity. Likewise, the *p*-chlorine is essential for high activity as demonstrated by the relative inactivity of the parent benzyl analog **1**. The position of the chlorine substituent is also important, since the *o*-chloro analog **3** is devoid of activity up to 200 mg/kg/day. Similarly, placement of the two chlorine atoms of **4** in the *o,o*-positions (**5**) abolishes activity. Addition of a chlorine to **4** to form the trichloride **6** also nearly abolishes activity.

L-153,153 (**4**) has shown significant suppressive activity against *T. cruzi* in 20 separate studies using the aforementioned protocol. Experiments were conducted with L-153,153 to obtain a dose response curve and determine the dosage at which 50% of the parasites were suppressed relative to control (SD_{50}). A representative series of experiments is provided in table 2. The SD_{50} value consistently fell between 1 and 3 mg/kg/day and was calculated to be approximately 1.75 mg/kg/day. When compared to nifurtimox, L-153,153 (**4**) was approximately 7.5-fold more active on the basis of SD_{50}.

Table 3 summarizes the results of the curative studies. All mice which received vehicle only were dead by day 46 while all mice which received the reference compound, nifurtimox, were alive but were positive for parasites. Two of the 7 mice (designated mouse 1 and mouse 2) which received L-153,094 remained negative for parasites through day 46. Although the blood from these mice was microscopically negative for parasites, only 1 of 4 mice receiving blood from mouse 1 and 2 of 4 mice receiving blood from mouse 2 remained negative for parasites upon microscopic examination. Thus none of those C_3H mice originally treated with either L-153,094 or nifurtimox were cured of the infection.

The data obtained from extensive studies with L-153,153 (**4**) and L-153,094 (**2**) against *T. cruzi* in mice indicates that both are highly active (about 7 times nifurtimox) with no detectable toxicity. These compounds are considered worthy of further development as potential therapies for Chagas' disease in man.

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