In Vitro and In Vivo Activities of Aminoadamantane and Aminoalkylcyclohexane Derivatives against Trypanosoma brucei

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We recently discovered (12) that the bloodstream form of the African trypanosome, Trypanosoma brucei, is sensitive to the anti-influenza virus drug rimantadine. In the present report we describe the trypanocidal properties of a further 62 aminoadamantane and aminocoxyhexane derivatives. Seventeen of the compounds were found to be more active than rimantadine, with four inhibiting growth in vitro of T. brucei by >90% at concentrations of 1 M. The most active derivative (1-adamantyl-4-amino-cyclohexane) was about 20 to 25 times more effective than rimantadine. We observed a correlation between structural features of the derivatives and their trypanocidal properties; hydrophobic substitutions to the adamantane or cyclohexane rings generally enhanced activity. As with rimantadine, the activity in vitro varied with the pH. T. brucei was more sensitive in an alkaline environment (including a normal bloodstream pH of 7.4) and less sensitive under acidic conditions. Tests for activity in vivo were carried out with a mouse model of infection with a virulent strain of T. brucei. Although the parasitemia was not eliminated, it could be transiently suppressed by >98% with the most active compounds tested. These results suggest that aminoadamantane derivatives could have potential as a new class of trypanocidal agents.

Vector control and other public health measures have a successful history of containing African trypanosomiasis (3). However, war, civil unrest, and economic problems have resulted in a breakdown of these interventions, and the estimated annual incidence is now 300,000 cases (25, 27). The causative agents of human trypanosomiasis are the tsetse fly-transmitted protozoan parasites Trypanosoma brucei gambiense (western and central Africa) and Trypanosoma brucei rhodesiense (eastern and southern Africa). In the bloodstream of infected individuals, antigenic variation by the parasite prevents elimination by the immune system (19, 20), and the development of a vaccine is not considered feasible. The drugs used to treat trypanosomiasis are unsatisfactory (6, 15). They all require hospitalization for their administration, are expensive, can fail to eradicate parasitemia, and often have toxic side effects. Melarsoprol, which is used against the advanced stage of the disease that occurs once trypanosomes have invaded the central nervous system, causes 5 to 10% patient mortality due to arsenic encephalopathy. The only other drug available for clinical use against this stage of the disease, difluoromethylornithine, has limited efficacy against T. brucei rhodesiense infections and is very expensive. In the absence of treatment, trypanosomiasis is fatal, and the development of new chemotherapeutic approaches is thus a priority.

We recently discovered (12) that the bloodstream form of T. brucei is highly susceptible to rimantadine (α-methyl-1-adamantane methylamine), a drug which is licensed for the treatment and prophylaxis of influenza A virus infection. Rimantadine is a derivative of amantadine (1-amino-adamantane), and both compounds share the cage-like configuration characteristic of adamantanes (see Table 1). Rimantadine and amantadine have many desirable properties as chemotherapeutic agents. They are inexpensive, can be taken orally, produce few side effects (8), and readily cross the blood-brain barrier (22). In addition, their pharmacokinetics in humans have been extensively investigated (9, 26); they are well absorbed from the gastrointestinal tract and have a plasma half-life of up to 36 h. The target of these drugs is the viral protein M2, which forms a tetrameric voltage-gated proton channel (17) and functions by translocating protons across the viral membrane into the viral core (5). This acidification process facilitates the release of viral RNA. Additionally, M2 acts as a trans-Golgi membrane component, elevating the pH of this acidic compartment and protecting hemagglutinin (H7 type) against premature conformational transition (23). Both drugs interact with the amino acid(s) within the amino-terminal portion of the M2 transmembrane region, leading to a blockage of the proton channel (16). Amantadine, which also has trypanocidal activity (12), is used to treat Parkinson’s disease, in which its effect is associated with an increase in extracellular levels of dopamine. This is thought to be mediated by blockage of the transmembrane channel in the N-methyl-D-aspartate receptor, which results in antagonism of receptor function (13).

Both rimantadine and amantadine have similar anti-influenza virus properties, whereas against trypanosomes, rimantadine is significantly more toxic (12). By inference, the structural modifications which differentiate the two compounds are responsible for this enhanced activity, either directly or indirectly. We therefore reasoned that other aminoadamantane derivatives may possess even greater trypanocidal activity and that an evaluation of such compounds would give an insight...
into the chemical features responsible for the activity. Here we report on the testing of 62 aminoadamantane and aminoalkylcyclohexane derivatives. Several of these displayed considerable activity in vitro and in vivo against the bloodstream form of *T. brucei*.

**MATERIALS AND METHODS**

Chemicals. The compounds tested were synthesized by Merz (Frankfurt, Germany) (21). Their identities and purities were verified (nuclear magnetic resonance and infrared imaging, elemental analysis, gas chromatographic and high-pressure liquid chromatographic analyses), and the data are on file at Merz. The structures of the most active compounds tested are shown in Tables 1 and 2.

Parasites and drug testing in vitro. Bloodstream-form *T. brucei* (strain 427) was cultured in 25-cm³ flasks at 37°C in modified Iscove's medium (pH 7.4) (10). To establish the extent of activity, parasites were grown for 3 days in the presence of test compounds (aminoadamantane or aminoalkylcyclohexane derivatives), and the concentrations which inhibited growth by 50% (IC₅₀) and 90% (IC₉₀) were determined. In these experiments, which were performed at least in triplicate, the densities of untreated cultures increased from 0.25 × 10⁷ to 4 × 10⁷ cells ml⁻¹. After determination of cell densities at each drug concentration with a hemocytometer (Weber Scientific International Ltd.), drug sensitivity was expressed as a percentage of growth of control cells.

Drug testing in vitro. Batches of five mice (CD1 strain) were inoculated intraperitoneally (i.p.) with 10⁵ bloodstream-form *T. brucei* (strain 427) parasites from an exponentially growing culture. Treatment was initiated 6 h later, and subsequent doses were injected as indicated in the legend to Fig. 2. The parasite levels in the mice with the resulting infection were determined by cell counting after dilution of tail blood in 0.85% ammonium chloride (24).

**RESULTS**

Testing in vitro. In a preliminary screening, bloodstream-form *T. brucei* was cultured for 3 days in growth medium at pH 7.4 (Materials and Methods) in the presence of aminoadamantane or aminoalkylcyclohexane derivatives at 5 μg ml⁻¹ (approximately 20 to 25 μM). A range of activities was observed with the 62 compounds tested. With the most active compounds, overnight incubation at this concentration resulted in the lysis of all cells in the culture. These derivatives were tested further to determine the IC₅₀ and IC₉₀. Several were found to have appreciable activities (Tables 1 and 2). In some cases, the activities were more than 10 times greater than that which had been observed with rimantadine (12). The results obtained with the 12 most active aminoadamantane derivatives and the 8 most reactive aminoalkylcyclohexane derivatives are listed in Tables 1 and 2, respectively.

Two of the more active aminoadamantane derivatives (compounds 2/242 and 2/238) have substituted amino groups at position 1 of the adamantane ring. These substitutions have a marginal effect on activity (cf. compounds 2/242 and 2/177, Table 1). The major determinant in activity of these derivatives seems to be the alkyl substitution at position 3 of the adamantane ring. Parasites were largely refractory to treatment with compound 2/193 (N-methyl-1-amino-3-methyl-adamantane). However, replacement of the 3-methyl group in compound 2/193 with isopropyl (compound 2/242) or n-butyl (compound 2/238) side chains significantly enhanced the trypansomoidal effect. In contrast, these modifications ablated the anti-influenza virus properties of 2/193 (21).

Several compounds with nonsubstituted amino groups attached to the 1 position of the adamantane ring (Table 1) displayed considerable activity against *T. brucei*. The effects of these adamantane derivatives were greatly enhanced by having bulky side chains at position 3. For example, compound 2/177 (1-amino-3-isopropyl-adamantane), which contains an isopropyl substitution at this position, is fivefold more active than amantadine. With compounds 2/180 and 2/182, which contain phenyl and cyclohexyl side groups, respectively, at the 3 position, the activity is increased by 9- and 49-fold, respectively. The presence of an additional cyclohexyl group at position 5 (compound 2/183) also slightly enhanced the trypansomoidal properties (Table 1). The most active compound of this group of derivatives and the most active compound tested overall was compound 2/146 (1-adamantyl-4-amino-cyclohexane), which had an IC₉₀ of 0.41 μM. This compound has an aminocyclohexyl group at position 1 on the adamantane ring, a feature that increases activity by 80-fold (Table 1). In contrast to this enhanced activity against trypanosomes, derivatives containing any of the substitutions described above have considerably reduced efficacy in anti-influenza virus assays compared to the efficacy of amantadine (21). As an instance of this, addition of the cyclohexyl side chain at position 3 of amantadine considerably enhances trypansomidal activity (compound 2/182; 1-amino-3-cyclohexyl-adamantane), whereas it reduces antiviral activity more than 20-fold (21).

Derivatives in which the amino group was attached to ethyl or propyl side chains at position 3 of the adamantane ring were also effective at inhibiting parasite growth (see data for compounds 2/15, 2/138, 2/23, and 2/173, Table 1). These activities, however, could also be dramatically affected by other substitutions on the adamantane ring. For example, replacement of the 1-methyl group in compound 2/15 with a hydroxyl largely negated the trypansomoidal properties. The effects of this substitution are to make the compound less hydrophobic and the amino group less basic. Derivatives with a hydroxyl group at this position have also been shown to lack significant activity in influenza virus assays (21). Substitution of the 1-methyl group in compound 2/15 with a phenyl group (2/173; 1-phenyl-3-ethyl-amino adamantane) enhanced activity against trypanosomes by three- to fourfold. This compound was one of the most active compounds tested (Table 1).

We also investigated the effects of a number of aminoalkylcyclohexane derivatives on cultured bloodstream-form *T. brucei* (Table 2). These compounds displayed a range of activities that were comparable to those of the aminoadamantane derivatives (Table 1). The most active of these compounds have IC₅₀ of approximately 1 μM and share structural similarities. Three of the derivatives have aminomethyl (compounds 2/662 and 2/644) or aminomethyl-propyl (compound 2/645) groups attached to the cyclohexane ring at position 1. They also have dimethyl substitutions at the 3 and 5 positions, a feature present in other active derivatives such as compounds 2/601 and 2/639 (IC₅₀, approximately 6 μM). It can also be inferred from our data that the presence of dipropyl side chains, as in compound 2/626 (Table 2), greatly enhances activity.

pH-dependence of activity. Previously, we noted that the trypansomidal activities of both rimantadine and amantadine are enhanced as the pH of the growth medium increases (12). We therefore examined whether this also occurred with the compounds tested in the present study. Bloodstream-form *T. brucei* was cultured in medium in which the pH ranged from 6.2 to 8.0 in the presence of different concentrations of five of the derivatives. The data obtained with compounds 2/15 (A) and 2/662 (B) are shown in Fig. 1. With increasing pH of the medium, the
TABLE 1. Susceptibility of cultured bloodstream-form *T. brucei* to aminoadamantane derivatives<sup>a</sup>

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>IC&lt;sub&gt;90&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/242</td>
<td><img src="image1" alt="Structure" /></td>
<td>6.48 ± 0.25</td>
<td>11.39 ± 0.86</td>
</tr>
<tr>
<td>2/177</td>
<td><img src="image2" alt="Structure" /></td>
<td>4.91 ± 1.21</td>
<td>8.07 ± 0.63</td>
</tr>
<tr>
<td>2/151&lt;sup&gt;b&lt;/sup&gt;</td>
<td><img src="image3" alt="Structure" /></td>
<td>5.25 ± 0.74</td>
<td>7.58 ± 0.20</td>
</tr>
<tr>
<td>2/180&lt;sup&gt;b&lt;/sup&gt;</td>
<td><img src="image4" alt="Structure" /></td>
<td>2.77 ± 0.38</td>
<td>3.75 ± 0.45</td>
</tr>
<tr>
<td>2/15</td>
<td><img src="image5" alt="Structure" /></td>
<td>2.14 ± 0.32</td>
<td>3.64 ± 1.07</td>
</tr>
<tr>
<td>2/238</td>
<td><img src="image6" alt="Structure" /></td>
<td>1.55 ± 0.27</td>
<td>2.52 ± 0.47</td>
</tr>
<tr>
<td>2/138&lt;sup&gt;b&lt;/sup&gt;</td>
<td><img src="image7" alt="Structure" /></td>
<td>1.15 ± 0.04</td>
<td>1.56 ± 0.04</td>
</tr>
<tr>
<td>2/23&lt;sup&gt;d&lt;/sup&gt;</td>
<td><img src="image8" alt="Structure" /></td>
<td>1.22 ± 0.09</td>
<td>1.48 ± 0.04</td>
</tr>
<tr>
<td>2/173&lt;sup&gt;b&lt;/sup&gt;</td>
<td><img src="image9" alt="Structure" /></td>
<td>0.62 ± 0.03</td>
<td>0.92 ± 0.07</td>
</tr>
<tr>
<td>2/182&lt;sup&gt;b&lt;/sup&gt;</td>
<td><img src="image10" alt="Structure" /></td>
<td>0.52 ± 0.04</td>
<td>0.70 ± 0.04</td>
</tr>
<tr>
<td>2/183&lt;sup&gt;d&lt;/sup&gt;</td>
<td><img src="image11" alt="Structure" /></td>
<td>0.37 ± 0.03</td>
<td>0.51 ± 0.03</td>
</tr>
<tr>
<td>2/146&lt;sup&gt;d&lt;/sup&gt;</td>
<td><img src="image12" alt="Structure" /></td>
<td>0.33 ± 0.04</td>
<td>0.41 ± 0.04</td>
</tr>
</tbody>
</table>

<sup>a</sup> Cultured bloodstream-form *T. brucei* was incubated at 37°C for 3 days in the presence of aminoadamantane derivatives (see Materials and Methods). Initially, each compound was screened at 5 µg ml<sup>-1</sup> to determine the inhibitory effect. The IC<sub>50</sub> and the IC<sub>90</sub> of the more active compounds were then evaluated. Each experiment was performed in triplicate unless indicated otherwise. Unless indicated otherwise, the compounds were dissolved in H<sub>2</sub>O. Control cells were treated accordingly. Values are presented as the mean ± the standard deviation from the mean.

<sup>b</sup> Stock compounds (20 mg ml<sup>-1</sup>) were dissolved in dimethyl sulfoxide.

<sup>c</sup> Data were obtained from six experiments.

<sup>d</sup> Stock compounds were dissolved in 50:50 (vol/vol) ethanol-H<sub>2</sub>O.
activity of each compound was significantly enhanced. Similar results were obtained with compounds 2/146, 2/173, and 2/182 (data not shown). For example, \textit{T. brucei} was largely refractory to treatment with 0.2 mg of compound 2/173 ml (0.7 mM) at pH 6.6 and 7.0, whereas in the pH range of 7.2 to 7.4, growth was inhibited by approximately 90%. Although the effects observed at nonphysiological pH should be interpreted with caution, our results suggest that these derivatives could share a common trypanocidal mechanism with rimantadine. Furthermore, it can be inferred that there may be similarities in the mechanisms by which the effects of aminoalkylcyclohexanes (compound 2/662) and aminoadamantanes are mediated.

\textbf{Testing in vivo.} We chose the CD1 mouse strain for in vivo testing of the derivatives. The immune system of these mice is unable to control a \textit{T. brucei} (strain 427) infection, which is generally lethal within 5 to 7 days. Initially we tested mice for their ability to tolerate 16 mg kg of body weight \textsuperscript{-1} administered i.p. three times daily. Compounds 2/626 and 2/645 exhibited toxicity, and their use was discontinued. Six compounds whose activities covered the range of activities detected in vitro were then selected for tests in vivo: compounds 2/146, 2/173, 2/138, 2/23, 2/15, and 2/238. The compounds were administered i.p. in six doses over 3 days following infection of mice with \num{10^8} parasites (see legend to Fig. 2 for details). Compounds 2/146 and 2/173 were the most effective at suppressing parasitemia (Fig. 2A). For up to 4 days following the cessation of treatment, the densities of the bloodstream form of the parasites was 50 to 200 times lower in the experimental mice than in the controls. A 10-fold reduction in the level of parasitemia could be observed with compounds 2/23 and 2/138 (Fig. 2B). Compounds 2/15 and 2/238 also had some transient effect on parasitemia, but the differences were not statistically significant (data not shown). Thus, the extent to which the level of bloodstream-form parasites was suppressed in mice by the various derivatives appears to parallel their activity in vitro (Table 1). Some deaths were observed among mice treated with compounds 2/146, 2/173, and 2/23 (Fig. 2). These were not associated with a high level of parasitemia. This may indicate a degree of toxicity associated with the levels and repeated doses used in these experiments.

\begin{table}
\centering
\caption{Susceptibility of cultured bloodstream-form \textit{T. brucei} to aminoalkylcyclohexane derivatives\textsuperscript{a}}
\begin{tabular}{llll}
\hline
Compound & Structure & IC\textsubscript{50} (\textmu M) & IC\textsubscript{90} (\textmu M) \\
\hline
2/639 & \includegraphics[width=0.2\textwidth]{table1化合物结构1} & 5.86 \pm 0.82 & 8.38 \pm 0.13 \\
2/601 & \includegraphics[width=0.2\textwidth]{table1化合物结构2} & 6.11 \pm 0.34 & 7.99 \pm 0.09 \\
2/614 & \includegraphics[width=0.2\textwidth]{table1化合物结构3} & 2.35 \pm 0.47 & 3.76 \pm 0.38\textsuperscript{b} \\
2/634 & \includegraphics[width=0.2\textwidth]{table1化合物结构4} & 2.59 \pm 0.59 & 3.50 \pm 0.68\textsuperscript{b} \\
2/662 & \includegraphics[width=0.2\textwidth]{table1化合物结构5} & 1.03 \pm 0.13 & 1.62 \pm 0.34\textsuperscript{b} \\
2/644 & \includegraphics[width=0.2\textwidth]{table1化合物结构6} & 1.05 \pm 0.09 & 1.41 \pm 0.14 \\
2/645\textsuperscript{c} & \includegraphics[width=0.2\textwidth]{table1化合物结构7} & 0.89 \pm 0.08 & 1.17 \pm 0.04 \\
2/626\textsuperscript{c} & \includegraphics[width=0.2\textwidth]{table1化合物结构8} & 0.95 \pm 0.04 & 1.11 \pm 0.04 \\
\hline
\end{tabular}
\textsuperscript{a} Cultured bloodstream-form \textit{T. brucei} was incubated at 37°C for 3 days in the presence of aminoalkylcyclohexane derivatives as described in the text (see Materials and Methods and Table 1). Each experiment was performed in triplicate unless indicated otherwise. Unless indicated otherwise, the compounds were dissolved in H\textsubscript{2}O. Control cells were treated accordingly.
\textsuperscript{b} Data were obtained from six experiments. Values are presented as means \pm standard deviations from the means.
\textsuperscript{c} Stock compounds (20 mg ml \textsuperscript{-1}) were dissolved in dimethyl sulfoxide.
\end{table}
DISCUSSION

We have identified 11 aminoadamantane derivatives (Table 1) and 6 aminoalkylcyclohexane derivatives (Table 2) which have greater trypanocidal activity than rimantadine (12). Our data provide insights into the structural features that confer this activity and should facilitate the design of compounds capable of inhibiting trypanosome growth at even lower concentrations. We also found that there was no general correlation between the effects of these compounds on trypanosomes (Table 1) and their anti-influenza virus activities (21).

An essential requirement for activity against T. brucei in the adamantane derivatives is possession of an amino group (Table 1). This can be attached directly to the adamantane ring or can be attached via a side chain at the 1 or the 3 position. The amino group can be substituted or nonsubstituted. In mammalian cells, the weakly basic aminoadamantane derivatives accumulate in lysosomes, where they act as amines and increase the intralysosomal pH. This is thought to account for the secondary anti-influenza virus activities of these compounds, although in the case of amantadine, this occurs only at concentrations 2 orders of magnitude greater than those used in the present study. The elevated pH blocks the conformational changes in hemagglutinin that are necessary for fusion of the viral and endosomal membranes. By analogy, one possible mechanism for the activities of these derivatives in trypanosomes is that they become concentrated in parasite lysosomes, where they function as weak bases and promote an increase in pH. However, compound 2/242, which has a secondary amino group, did not have greater trypanocidal activity than a similar but less basic compound containing a primary amino group (compound 2/177, Table 1). It also appears that the adamantane cage-like configuration is not in itself a prerequisite for activity. Compounds in which the amino group is attached only to a cyclohexane ring also exhibited considerable toxicity to trypanosomes (Table 2).

The trypanocidal properties of amantadine were significantly enhanced by the addition of a bulky side group at position 3. A cyclohexane ring was the most effective of these substitutions (compound 2/182, Table 1), suggesting that increased hydrophobicity may be an important factor in determining the activity. The effects of similar substitutions on the properties of rimantadine will be of interest given that rimantadine itself has been shown to be considerably more active than amantadine (12). There was also a significant correlation between hydrophobicity and trypanocidal activity with aminoalkylcyclohexanes and with aminoadamantane derivatives in which the amino group is attached to either ethyl or propyl side chains. The most active of the compounds that we tested (compound 2/146, Table 1) was distinctive in having an amino-cyclohexyl group attached at the 1 position. These results suggest that derivatives of compound 2/146, modified by the addition of alkyl groups to the adamantane ring, could have even greater trypanocidal activities.

Determination of the mechanisms of action of the aminoadamantane derivatives and identification of the target in trypanosomes will be of considerable importance from a drug design perspective. Such information would complement the observations described in this report as regards the correlation between the structural features of the derivatives and their activities. The predominant anti-influenza virus properties of both rimantadine and amantadine are mediated by their channel-blocking activities (16). The interaction between the drugs and the M2 protein is highly specific, and single-amino-acid replacements between positions 27 and 31 can confer resistance (4, 7). One possible explanation for the trypanocidal activities of the aminoadamantane derivatives, by comparison with the situation in influenza virus-infected cells, is that these compounds target an essential T. brucei membrane-localized ion channel or transporter. This could account for the enhanced activities of the more hydrophobic derivatives. T. brucei is an extracellular bloodstream parasite. In humans the most critical phase of an infection occurs when the parasite invades the central nervous system. Therefore, important parameters in the efficacies of trypanocidal drugs are their half-

FIG. 1. Susceptibility of bloodstream-form T. brucei to derivatives at different pHs. Parasites were cultured for 3 days in growth medium (see Materials and Methods) in the presence of a number of different pHs in the range of 6.2 to 8.0 and in the presence or absence of derivatives, as indicated. Cell densities were determined by hemocytometer counting. Values are expressed as a percentage of the growth obtained at the optimal pH in the absence of treatment. *, control cells; ⊙, cells cultured in the presence of the corresponding compounds at 750 ng ml\(^{-1}\) (4.00 μM) (A) or 300 ng ml\(^{-1}\) (1.30 μM) (B); •, cells cultured in the presence of 1,000 ng ml\(^{-1}\) (5.35 μM) (A) or (B) 500 ng ml\(^{-1}\) (2.15 μM) (B).
lives, their concentrations in serum, and their ability to cross the blood-brain barrier. Pharmacokinetic data are not available for most of the compounds used in the study described in this report, but both amantadine and rimantadine have been well studied. Steady-state levels in serum of 0.5 to 1.0 μg ml⁻¹ (2.5 to 5.0 μM) and above and serum half-lives of between 24 and 36 h have been widely reported (1, 2, 9, 14, 26). In addition, the drugs readily gain access to the central nervous system (22). It is likely that the chemically related aminoadamantane derivatives used in the present study will have similar properties. In these circumstances, some of the compounds would have the potential to eliminate a human infection even when the disease is at an advanced stage. For preliminary testing in vivo, we used a mouse model, although this represented an extremely stringent experimental system. The half-life of rimantadine in the serum of mice is only 1.5 h (11), and much higher doses of the drug must be administered to attain the same concentration that is achievable in the serum of humans. Despite this, we were to detect a significant suppression of parasitemia in mice, particularly with compounds 2/146 and 2/173 (Fig. 2A). Parasite levels did increase following the cessation of treatment, and there was also evidence of toxicity at the concentrations used (six injections, each at 16 mg kg⁻¹, over 3 days). However, this dosage is more than 10 times that required in human adults to achieve a steady-state serum rimantadine level of 2 μM (with an oral dose of 100 mg twice daily) (26).

The results described in this report provide a strong case for further studies on the potential of selected aminoadamantane derivatives as treatments for African trypanosomiasis. We have identified structural features that are important for activity and demonstrated that some derivatives have an in vivo effect in mice. It will now be important to extend this investigation to larger nonrodent animals, in which the course of the disease and the pharmacokinetics more closely reflect those in humans. The use of domestic animals may be a useful way forward. Cattle are natural hosts of *T. brucei* and the closely related parasites *Trypanosoma congolense* and *Trypanosoma evansi*. These infections are of considerable economic significance. Another closely related trypanosome of veterinary importance is the equine parasite *Trypanosoma equiperdum*. Serum rimantadine concentrations of 10 μM have been reported to be achievable in horses (18), suggesting that this may also represent a suitable model for in vivo testing.

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