Structurally Simple Inhibitors of Lanosterol 14α-Demethylase Are Efficacious In a Rodent Model of Acute Chagas Disease

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We report structure—activity studies of a large number of dialkyl imidazoles as inhibitors of Trypanosoma cruzi lanosterol-14α-demethylase (L14DM). The compounds have a simple structure compared to posaconazole, another L14DM inhibitor that is an anti-Chagas drug candidate. Several compounds display potency for killing T. cruzi amastigotes in vitro with values of EC50 in the 0.4–10 nM range. Two compounds were selected for efficacy studies in a mouse model of acute Chagas disease. At oral doses of 20–50 mg/kg given after establishment of parasite infection, the compounds reduced parasitemia in the blood to undetectable levels, and analysis of remaining parasites by PCR revealed a lack of parasites in the majority of animals. These dialkyl imidazoles are substantially less expensive to produce than posaconazole and are appropriate for further development toward an anti-Chagas disease clinical candidate.

Introduction

Chagas disease causes the third largest parasitic disease burden in the world and the largest in the Western hemisphere, currently affecting 16–18 million people throughout Central and South America.1 The disease is caused by the parasite Trypanosoma cruzi (T. cruzi), which is able to invade a wide variety of host cells. The vaccine prospects for preventing Chagas disease are not promising because the pathogen has developed complex immune evasion techniques to allow for persistent infection.2 Drug therapy options for Chagas disease are limited. The principal drugs are benznidazole and nifurtimox, which have modest efficacy during the acute phase of the disease and are not effective for treatment of the chronic, life-threatening stage. Both of these nitroheterocyclic drugs are poorly tolerated by adults. In short, more effective and better-tolerated anti-Chagas disease drugs are greatly needed.3

Sterol biosynthesis is a complex enzymatic pathway, which produces membrane lipids for many eukaryotic organisms. Mammals produce cholesterol as their primary sterol, fungi produce ergosterol, and T. cruzi produces a mixture of ergosterol-like sterols that contain various alkyl substituents at C24.4 Sterol synthesis in mammals, yeast, and T. cruzi go through the common intermediate lanosterol, which is formed in several steps from acetyl-CoA. The first of the postlanosterol processing steps is the removal of the methyl group at C14 by lanosterol 14α-demethylase (cytochrome P450 subfamily 51) (L14DM). Because the parasite apparently cannot survive solely on cholesterol salvaged from its host, enzymes of the parasite sterol biosynthetic pathway offer potential targets for the development of drugs. Urbina and others have shown that a number of inhibitors of fungal L14DM, including the recently developed antifungal drug posaconazole, are potent inhibitors of T. cruzi L14DM and are able to cure mice suffering from acute and chronic Chagas disease.5 The use of posaconazole to treat Chagas disease is being considered, however its manufacturing costs may limit widespread use especially in the long term treatment of the chronic disease.5

In our studies of inhibitors of protein farnesyltransferase as antiparasite agents such as 1 (Figure 1), we found that compounds such as 2, which lack the methionyl group, do not inhibit parasite protein farnesyltransferase and yet display potent activity in blocking the growth of T. cruzi amastigote stage (intracellular) parasites.6 Further studies showed that compounds in this class led to the accumulation of lanosterol in amastigotes and to the formation of unusual sterols that are predicted to result from the blockade of the lanosterol 14α-demethylation step.7 Compounds in this class were also found to bind to recombinant T. cruzi L14DM, causing a spectral shift that is consistent with coordination of the unsubstituted imidazole nitrogen to the heme iron of this cytochrome P450.7 Because the methyl ester of 2 is rapidly hydrolyzed by enzymes in serum, we went on to prepare derivatives including 3 (Figure 1) that displayed desirable pharmacokinetic properties in mice.8 Administration of a single dose of 3 to mice at 30 mg/kg by oral gavage led to a maximal drug concentration in the plasma of 16 μM after 1 h, and 3 was lost with a serum half-life of ~4 h. Administration of 3 at 50 mg/kg, twice per day for 14 days, to T. cruzi-infected mice reduced parasitemia in blood to ~1% of the level seen in untreated mice.7 These encouraging results along with the low cost of goods anticipated for this new class of L14DM inhibitors, which lack stereogenic centers, prompted us to carry out extensive structure—activity studies of 3 in an effort to maximize potency against T. cruzi amastigotes while maintaining respectable pharmacokinetic properties. The synthesis of analogues of 3 was guided using...
a model of the structure of *T. cruzi* L14DM templated on the X-ray structure of L14DM from *Mycobacterium tuberculosis*.

The present article discloses the synthesis and structure–activity relationship studies of a series of dialkyl substituted imidazole derivatives as potent L14DM inhibitors for Chagas chemotherapy.

**Results and Discussion**

**Chemistry.** Synthetic procedures are illustrated in Schemes 1–7. Scheme 1 shows the synthesis of dialkyl imidazoles containing a methoxycarbonyl group and also shows the synthetic steps used to make most of the compounds reported in this study, the first of which is alkylation of the N-tritylated imidazole carboxaldehyde 4 to give the N-alkylated imidazole 5. The second piece, the substituted aniline 9, contains a phenyl–phenyl bond, which is made using the Suzuki cross coupling, followed by standard functional group transformation. The two inhibitor pieces are joined by reductive amination to give 10a–l.

Scheme 2 shows the preparation of dialkyl imidazoles containing additional acyl groups (esters and amides). It is based on standard functional group transformations and the general methods outlined in Scheme 1. Dialkyl imidazoles containing a ketone functional group are shown in Scheme 3. After several unsuccessful attempts, we found that the Fries rearrangement worked well to install the ketone group. The phenolic hydroxyl group was conveniently activated as a triflate ester to generate 18a–c, followed by the usual Suzuki cross coupling to produce 19a–c.

Non acyl-containing functional groups were introduced into the inhibitor scaffold in place of the methoxycarbonyl group using a Sandmeyer reaction (22b,c, Scheme 4a) by heating the aryl halide with the secondary amine (22d,e) or reductive amination with formaldehyde (22f). Incorporation of small heterocycles into the dialkyl imidazole scaffold is shown in Scheme 4b using the appropriate aryl bromide in the presence of KOAc and catalytic Pd(PPh3)4 in DMAC.

Preparation of dialkyl imidazoles containing the benzothiazole appendage is shown in Scheme 5. The key reaction is treatment of the aryl bromide with benzothiazole in the presence of KOAc and catalytic Pd(PPh3)4 in DMAC. Preparation of dialkyl imidazoles containing the benzothiazole appendage in Scheme 5. The key reaction is treatment of the aryl bromide with benzothiazole in the presence of KOAc and catalytic Pd(PPh3)4 in DMAC. The latter proceeded in poor yield, so we developed a second method starting with benzoic acid derivative 7, which was converted to the acid chloride and then condensed with 2-amino-thiophenol using pyridine in xylene. The addition of PTSA to the reaction mixture considerably improved the overall yield up to 70%.

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**Scheme 1. Synthesis of the Dialkylimidazoles Containing the Methoxycarbonyl Group**

![Scheme 1](image-url)
Scheme 6a shows the synthesis of additional dialkyl imidazoles containing different substituents on each of the phenyl groups using methods derived mainly from Scheme 1. Scheme 6b shows the route to a dialkyl imidazole containing a 3,4-diphenyl unit, which was installed via Suzuki cross coupling. Finally, Scheme 7 shows the route for preparing dialkyl imidazoles with an ortho-amino group on the benzyl unit attached to the imidazole nitrogen. Several compounds in this series were prepared as we found that the addition of this amino group improved antiparasite potency. The route is derived from Scheme 1. We also developed a large scale synthesis of 44a and 44d. In this case, all reaction products were purified by recrystallization except for 41 and 42.

Molecular Modeling and Structure–Activity Relationship Studies. We made use of the homology model of the T. cruzi L14DM in complex with tipifarnib based on the M. tuberculosis enzyme structure earlier described. Design and docking studies were carried out with the FLO/QXP program suite, version 0602. In each case, amino acid residues within 11 Å of tipifarnib were included in the binding site model for Metropolis Monte Carlo searches and energy minimization procedures. Details of the procedures were earlier described.

To understand the structure–activity relationship of the various modifications of the dialkyl imidazoles, we docked the various compounds into the homology model of the T. cruzi L14DM. The scaffold of all compounds in this paper consists of a dialkyl imidazole, where one substituent is benzyl or biphenyl and the other is biphenylamine. The imidazole nitrogen binds to the heme iron, and the two substituents occupy mainly hydrophobic clefts. The anilino fragment of the scaffold is surrounded by several hydrophobic residues, Tyr 77, Phe 84, and Ala 265, but the amino group does not interact directly with the enzyme, thus acting as a spacer.

To study the effect of substitutions on biological activity, we explored the possibility with various functional groups by varying the size, polarity, and position on the phenyl ring (Table 1). Compounds described in this report were generated from previous compounds shown to have potent activity against T. cruzi cells.

The benzyl substituent on the imidazole fits in a hydrophobic cleft created by Phe 264, Leu 330, and Val 435 and allows for small substituents in the ortho and meta positions only on one side of the aromatic ring and for larger substituents in the para position. Small meta substituents (10c, d, e) also contact Val 435. Various hydrophobic para substituents are tolerated (10c, d, e, h) consistent with the lipophilic character of the cleft’s extension defined by Tyr 77, Met 80, Ile 183, and Met 434. Not surprisingly, the p-phenyl substituent (10h) is the most potent, with an EC50 of 0.5 nM because of its size. The t-butyl substitution (10k) leads to poor activity, as it is too bulky for the narrow cleft. Hydrophilic substituents in the para position (10b, i) are incompatible with the...
Scheme 4. (a) Synthesis of Dialkylimidazoles Containing Non-Acyl Functional Groups; (b) Synthesis of Dialkylimidazoles Containing Heterocycles*  

Reagents and conditions: (a) (i) Br₂, AcOH, rt, 1 h, 76%; (ii) PhB(OH)₂, Pd(OAc)₂, K₂CO₃, acetone/H₂O, reflux, 86%. (b) NaNO₂, H₂SO₄, AcOH; (i) CuBr₂, HCl, 71%; (ii) CuCl₂, HCl, 75%. (c) Zn(CN)₂, Pd(PPh₃)₄, DMF, 100 °C, overnight, 60–65%. (d) Piperidine, 100 °C, 2 h, 90%. (e) Pyrrolidine, 80 °C, 2–3 h, 80–90%. (f) HCHO, H₂SO₄, NaBH₄, THF, 10–30 °C, 0.5 h, 65–70%. (g) SnCl₂·2H₂O, EtOAc, reflux, 6 h, 76–80%. (h) AcOH, 5, MeOH, 4 Å mol sieves, NaBH₃CN, overnight, rt, 60–65%. (i) Heterocycle, Pd(PPh₃)₄, KOAc, DMAC, 160 °C, overnight, 32–35%.

Scheme 5. Synthesis of Dialkylimidazoles Containing a Benzothiazole Group*  

Reagents and conditions: (a) PhR₁B(OH)₂, Ba(OH)₂·8H₂O, Pd(PPh₃)₄, DME-H₂O (5:1), reflux, 90%. (b) NaNO₂, H₂SO₄, AcOH, CuBr₂, HCl, 71%. (c) Benzothiazole, Pd(PPh₃)₄, KOAc, DMAC, 160 °C, overnight, 32–35%. (d) SnCl₂·2H₂O, EtOAc, reflux, 6 h, 76–80%. (e) AcOH, 5, MeOH, 4 Å mol sieves, NaBH₃CN, overnight, rt, 60–65%.
lipophilic character of the cleft. Only one ortho substituent appears to be tolerated (10i), projecting an amino group toward His 268 and making a hydrogen bond with the imidazole. Hydrogen bond acceptors (10b) or hydrophobic groups (10c,d,e) in the ortho position would desolvate the imidazole and are expected to have very poor activity.

We achieved considerable potency in the ester series of analogues (Table 1), but these compounds lack metabolic...
stability in rodents because of hydrolases that are prevalent both extra- and intracellularly. Schematic changes have been made on the basic skeleton by docking into the homology model. On the basis of the previous data (Table 1), we decided to maintain the biphenyl group on the imidazole and modify the ester functionality. Our first approach involved the synthesis of various esters and amides in anticipation of reducing the risk of plasma hydrolysis. We further explored the series and undertook a systematic survey of structure—activity relationships.

Various substituents coming off the para position of the anilino fragment of the scaffold, various residues lining the hydrophobic cleft (Table 3). As to the meta position, the benzothiazole from the previous compounds and assessed their viability by docking into the homology model. On the basis of the previous data (Table 1), we decided to maintain the biphenyl group on the imidazole and modify the ester functionality. Our first approach involved the synthesis of various esters and amides in anticipation of reducing the risk of plasma hydrolysis. We further explored the series and undertook a systematic survey of structure—activity relationships.

Various substituents coming off the para position of the anilino fragment in the scaffold (Table 2) can make limited extra contacts and afford a slightly better activity. These structural differences do not significantly affect the binding activity relationships.

It is not surprising that the methyl group of the previous compounds is relatively ineffective against cultured T. cruzi (Table 2). In the hope of maintaining these improved pharmacokinetic properties, we focused on further modifications to increase potency. In particular, the benzothiazole from the structure—activity study in Table 2 was retained and the extra- and intracellularly. Schematic changes have been made on the basic skeleton by docking into the homology model. On the basis of the previous data (Table 1), we decided to maintain the biphenyl group on the imidazole and modify the ester functionality. Our first approach involved the synthesis of various esters and amides in anticipation of reducing the risk of plasma hydrolysis. We further explored the series and undertook a systematic survey of structure—activity relationships.

Table 1. Activities of Compounds Showing General Structure I against T. cruzi Amastigotes and Murine Fibroblast Cells (Scheme 1)

<table>
<thead>
<tr>
<th>compd</th>
<th>R</th>
<th>T. cruzi EC50 (nM) fibroblasts EC50 (nM)</th>
<th>meta T. cruzi EC50 (nM) fibroblasts EC50 (nM)</th>
<th>ortho T. cruzi EC50 (nM) fibroblasts EC50 (nM)</th>
</tr>
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<tbody>
<tr>
<td>10a</td>
<td>H</td>
<td>80</td>
<td>&gt;1000</td>
<td>30</td>
</tr>
<tr>
<td>10b</td>
<td>NO2</td>
<td>100</td>
<td>&gt;10000</td>
<td>20</td>
</tr>
<tr>
<td>10c</td>
<td>CH3</td>
<td>5</td>
<td>50000</td>
<td>20</td>
</tr>
<tr>
<td>10d</td>
<td>Cl</td>
<td>5</td>
<td>25000</td>
<td>20</td>
</tr>
<tr>
<td>10e</td>
<td>Br</td>
<td>20</td>
<td>&gt;10000</td>
<td>20</td>
</tr>
<tr>
<td>10f</td>
<td>OMe</td>
<td>25</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10g</td>
<td>CN</td>
<td>25</td>
<td>&gt;10000</td>
<td>100</td>
</tr>
<tr>
<td>10h</td>
<td>Ph</td>
<td>0.5, 0.9, 1.0</td>
<td>&gt;1000</td>
<td>ND</td>
</tr>
<tr>
<td>10i</td>
<td>NH2</td>
<td>250</td>
<td>&gt;10000</td>
<td>130</td>
</tr>
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<td>10j</td>
<td>F</td>
<td>ND</td>
<td>ND</td>
<td>150</td>
</tr>
<tr>
<td>10k</td>
<td>C(CH3)3</td>
<td>ND</td>
<td>ND</td>
<td>475</td>
</tr>
<tr>
<td>10l</td>
<td>2,4-F2</td>
<td>21, 25</td>
<td>&gt;1000</td>
<td></td>
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</tbody>
</table>

* Multiple values for the same compound indicate independent determinations.

Table 2. Activities of Compounds Showing General Structure II against T. cruzi Amastigotes and Murine Fibroblast Cells (Scheme 2,3,4)

<table>
<thead>
<tr>
<th>compd</th>
<th>R</th>
<th>T. cruzi EC50 (nM)</th>
<th>T. cruzi EC50 (nM) Fibroblasts (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15a</td>
<td>COOEt</td>
<td>10</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>15b</td>
<td>COOiPr</td>
<td>50</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>15c</td>
<td>COOOcy</td>
<td>10</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>16a</td>
<td>C(=O)NHCH3</td>
<td>2,4, 3.0, 3.8, 5.1</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>16b</td>
<td>C(=O)N(CH3)2</td>
<td>9.3, 14</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>16c</td>
<td>C(=O)N(CH3)2</td>
<td>24, 39</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>20a</td>
<td>C(=O)CH3</td>
<td>40</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>20b</td>
<td>C(=O)Et</td>
<td>40</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>20c</td>
<td>C(=O)Pr</td>
<td>40</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>24b</td>
<td>Cl</td>
<td>6, 7</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>24c</td>
<td>CN</td>
<td>1.3, 1.8</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>24d</td>
<td>1-piperidine</td>
<td>23, 35</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>24e</td>
<td>1-pyrrolidine</td>
<td>22, 41</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>24f</td>
<td>N(CH3)2</td>
<td>20, 26</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>24g</td>
<td>OCH3</td>
<td>7, 9</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>27a</td>
<td>5-thiazole</td>
<td>9, 9, 22, 28, 28</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>27b</td>
<td>2-pyrole</td>
<td>100</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>27c</td>
<td>2-benzoferan</td>
<td>40</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>27d</td>
<td>2-benzoazole</td>
<td>19, 7, 15, 14, 22, 21</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>27e</td>
<td>2-benzoxazole</td>
<td>100</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>27f</td>
<td>3-benzoxazole</td>
<td>10</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>27g</td>
<td>3-anthranil</td>
<td>50</td>
<td>&gt;10000</td>
</tr>
</tbody>
</table>

For compounds shown in Table 2, they are not packed by the other residues Arg 96 and Met 97. It is not surprising that the methyl group of 32g and 32h can make extra contacts and afford a slightly better activity. These structural differences do not significantly affect the binding behavior, as shown in Table 3.
Table 3. Activities of Compounds Showing General Structure III against T. cruzi Amastigotes and Murine Fibroblast Cells (Scheme 5)

<table>
<thead>
<tr>
<th>compd</th>
<th>R</th>
<th>R1</th>
<th>EC50 T. cruzi (nM)</th>
<th>EC50 fibroblasts (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>32a</td>
<td>p-isopropyl</td>
<td>H</td>
<td>540, 600</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>32b</td>
<td>p-ethyl</td>
<td>H</td>
<td>110, 190</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>32c</td>
<td>p-chloro</td>
<td>H</td>
<td>250</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>32d</td>
<td>ap-difluoro</td>
<td>H</td>
<td>420, 630</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>32e</td>
<td>p-methyl sulfonyl</td>
<td>H</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>32f</td>
<td>p-(2-tolyl)</td>
<td>H</td>
<td>120, 180</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>32g</td>
<td>p-Ph</td>
<td>2-methyl</td>
<td>31, 42</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>32h</td>
<td>p-Ph</td>
<td>2,3-dimethyl</td>
<td>64, 101</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

Table 4. Activities of Compounds Showing General Structure IV against T. cruzi Amastigotes (EC50) and Murine Fibroblast Cells (nM) (Scheme 6)

<table>
<thead>
<tr>
<th>compd</th>
<th>R</th>
<th>R1</th>
<th>EC50 T. cruzi (nM)</th>
<th>EC50 fibroblasts (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35a</td>
<td>NO3</td>
<td>3-Ph</td>
<td>100</td>
<td>10000</td>
</tr>
<tr>
<td>35b</td>
<td>CH3</td>
<td>3-Ph</td>
<td>100</td>
<td>10000</td>
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<tr>
<td>35c</td>
<td>Cl</td>
<td>3-Ph</td>
<td>80</td>
<td>10000</td>
</tr>
<tr>
<td>35d</td>
<td>Ph</td>
<td>3-Ph</td>
<td>10, 29, 23, 20</td>
<td>10000</td>
</tr>
<tr>
<td>35e</td>
<td>H</td>
<td>3-Ph</td>
<td>360, 400</td>
<td>&gt;750</td>
</tr>
<tr>
<td>35f</td>
<td>H</td>
<td>3-choline 2</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>35g</td>
<td>H</td>
<td>(o-tolyl)</td>
<td>230, 310</td>
<td>&gt;1000</td>
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<tr>
<td>35h</td>
<td>H</td>
<td>3-(m-tolyl)</td>
<td>550</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>35i</td>
<td>H</td>
<td>3-(o,m-dimethyl phenyl)</td>
<td>260, 300</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>35j</td>
<td>H</td>
<td>3-(3′-pyridyl)</td>
<td>600, 760</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>35k</td>
<td>H</td>
<td>3-(4′-pyridyl)</td>
<td>520, 510</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>35l</td>
<td>Ph</td>
<td>4-COOCH3</td>
<td>80</td>
<td>&gt;10000</td>
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<td>35m</td>
<td>Ph</td>
<td>2-Ph</td>
<td>80</td>
<td>&gt;10000</td>
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<td>35n</td>
<td>Ph</td>
<td>4-Ph</td>
<td>200</td>
<td>&gt;10000</td>
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<tr>
<td>35o</td>
<td>Ph</td>
<td>3-Ph, 4-Ph</td>
<td>80</td>
<td>&gt;10000</td>
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Using the structure–activity from Table 1, we decided to explore further combinations of substitutions of the two-phenyl rings of the scaffold in the absence of the methyl ester substituent. Para substitutions on the benzyl recapitulate our findings from Table 1, with 35d being the most active. None of the substitutions on the anilino fragment were as active as the m-phenyl. Finally we combined the most active substituents of Tables 1–4. All compounds exhibit EC50s against T. cruzi in the 1–25 nM range (Table 5). We focused our attention toward the ortho amino substituted compound 10i, which is the analogue in this series showing the best potency against cultured T. cruzi. The combination of these observations resulted in a series of compounds possessing inhibition in the 1–2 nM range against T. cruzi (Table 5), and these structural differences significantly affect the potency. Among these the most active is 44d, which projects the amino-substituted biphenyl in the hydrophobic cleat that has a uniquely placed histidine for forming a hydrogen bond. It also possesses a benzothiazole that makes hydrophobic interactions with Tyr 77 and Phe 84. The predicted binding mode is shown in Figure 2.

**Binding of 44a and 44d to Recombinant T. cruzi L14DM in Vitro.** We tested the binding of key compounds 44a and 44d to recombinant T. cruzi L14DM by monitoring the difference in the visible spectrum that occurs when the imidazole nitrogen coordinates to the heme iron (Soret band shift) (Figure 3). Both compounds were found to bind tightly to L14DM, with a maximal difference spectrum obtained when the amount of inhibitor approached the total amount of enzyme in solution. This indicates that the equilibrium dissociation constant for the L14DM-inhibitor complex is ≈2.2 μM, the concentration of enzyme used in the assay. It was not possible to obtain accurate values of the dissociation constants because the use of lower enzyme concentrations gives rise to spectral signals that are not significantly above the noise. The spectral shift observed (type II difference spectrum7) is consistent with direct coordination of the imidazole nitrogen of the inhibitors to the heme-iron. Thus, it is expected that these compounds compete with lanosterol for binding to L14DM, but this was not established with kinetic studies.

**Pharmacokinetics and Activity of Compounds in the Murine Model of Chagas Disease.** Two compounds with potent activity against T. cruzi in vitro, 44a and 44d, were subjected to single dose (50 mg/kg by oral gavage) pharmacokinetic analysis. Both compounds showed good oral bioavailability, with maximum plasma concentrations achieved within 1–2 h post-dose. Over the next 24 h, the compounds exhibited sustained plasma concentrations, which were sufficient to maintain effective inhibition of T. cruzi L14DM. The plasma concentrations of 44a and 44d were found to be comparable, with 44d showing slightly lower clearance, indicating potential for increased exposure over time. These findings suggest that 44a and 44d are highly efficacious against T. cruzi L14DM in vivo, with promising pharmacokinetic profiles that support further clinical development.

**Figure 2.** Homology model of T. cruzi L14DM in complex with 44d.
The half-life is in excess of 3 h for each compound, showing that this compound is more stable than posaconazole of 7 days. Kinetic studies on Balb/c mice in groups of three. Table 6 gives the data summary, and plasma concentration-versus-time plots for each mouse are provided as Supporting Information. For 44a, the peak average blood level of 2.4 μg/mL (C_max) was obtained in 0.8 h (T_max) (average from 3 mice). Values for 44d are C_max = 7.2 μg/mL and T_max = 1.7 h. We collected plasma drug concentration data out to 5 h and obtained AUC_0–5h of 6.3 and 28.7 μg·h/mL for 44a and 44d, respectively. Accurate terminal elimination half-lives were not obtained, but the data show that the half-life is in excess of 3 h for 44a (Supporting Information). For 44d, significant drug loss was not observed out to 5 h (Supporting Information), showing that this compound is more stable than 44a in mice. These values are not very different from the published terminal phase half-life of posaconazole at 7–9 h in mice and rats.22 A long elimination half-life, as exhibited by these compounds, is believed to be important for successful elimination of the slowly dividing T. cruzi during chronic infection.23

The efficacies of the 44a and 44d in the murine model of Chagas disease were compared to vehicle and posaconazole (Figure 4). The compounds or vehicle were administered to a group of 6 mice at the indicated doses twice per day for 21 consecutive days to mice by oral gavage from days 7–27 postinfection. Parasitemia was monitored by microscopic examination of blood through 97 days postinfection. All mice in the vehicle group developed overwhelming parasitemia and were dead by day 16. Dramatic suppression of parasitemia was observed in all compound treated groups, with 44d at 50 mg/kg showing the most suppression along with posaconazole. The mice tolerated all of the treatments without apparent side effects.

Table 7. Mice Parasitemia by PCR

<table>
<thead>
<tr>
<th>compd</th>
<th>dose (mg/kg)</th>
<th>PCR parasite detection after 100 days post infection (no. of mice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>44d</td>
<td>50</td>
<td>2 of 6</td>
</tr>
<tr>
<td>44d</td>
<td>20</td>
<td>5 of 6</td>
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<tr>
<td>44d</td>
<td>50</td>
<td>6 of 6</td>
</tr>
<tr>
<td>posaconazole</td>
<td>20</td>
<td>0 of 6</td>
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</tbody>
</table>

Efficacy of compounds in mice infected with T. cruzi. Mice in groups of six were given compounds or vehicle by oral gavage twice per day from days 7–27 postinfection. Parasitemia was quantified on wet mounts of fresh blood. All vehicle treated mice were dead by day 16. Dramatic suppression of parasitemia was observed in all compound treated groups, with 44d at 50 mg/kg showing the most suppression along with posaconazole. The mice tolerated all of the treatments without apparent side effects.

Conclusions

From this study have come several new inhibitors of T. cruzi L14DM that are among the most potent inhibitors of in vitro T. cruzi amastigote growth known to date (subnanomolar to low nanomolar potency). Two of the most promising compounds,
44a and 44d, were chosen for studies in mice and display excellent pharmacokinetic properties including oral activity and reasonable stability in mouse plasma. These two compounds are efficacious in a mouse model of acute Chagas disease with efficacy comparable to that of posaconazole. This new class of L14DM inhibitors should be much cheaper to produce than posaconazole. Further studies are in progress to explore the animal toxicology profile of these compounds, as well as their efficacy in a chronic model of Chagas disease in mice, and efficacy studies in larger animals.

Experimental Section

Synthesis of Compounds. Unless otherwise indicated, all anhydrous solvents were commercially obtained and stored under nitrogen. Reactions were performed under an atmosphere of dry nitrogen in oven-dried glassware and were monitored for completeness by thin layer chromatography (TLC) using silica gel 60 F-254 (0.25 mm) plates with detection with UV light. 1H NMR spectra were recorded on dilute solutions in CDCl3, CD3OD, or DMSO-d6 at 300 MHz. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane (TMS). Coupling constants (J) are reported in Hz. Electron spray ionization mass spectra were acquired on a Bruker Esquire LC00066.

Flash chromatography was carried out with silica gel (40–63 µm). Preparative reverse phase HPLC was performed on an automated Varian Prep Star system using a gradient of 20% MeOH to 100% MeOH (with 0.1% trifluoroacetic acid) at 12 mL/min over 100 min. The crude product was purified by preparative HPLC to give a single noncompound peak (estimated purity at least 95%).

General Procedure for the Synthesis of 3-Alkyl-3-imidazole Carboxaldehyde (4). To a 1 L three-necked round-bottomed flask with an addition funnel was added 3H-imidazole-4-carboxaldehyde (12 g, 0.125 mol), trityl (3.2 g, 15 mmol) was dissolved in 15 mL of pyridine and 30 mL of water (400 mL) were added. The slurry was stirred for 30 min at 25 °C and stirred overnight under nitrogen. The reaction mixture was stirred at rt for 20 h. Hexane (40 mL) was added dropwise over 20 min. After the addition was complete, the mixture was stirred at rt to give a slurry. Triethylamine (30 mL, 0.215 mol) was added. After 5 min, NaCNBH3 (0.64 mmol) was added in portions. Under nitrogen for 1 h, after which acetic acid (4.4 mmol) was added. After 5 min, NaCNBH3 (0.64 mmol) was added in portions. The mixture was stirred at rt under nitrogen overnight. The reaction mixture was mixed with CH2Cl2 and saturated NaHCO3. The organic layer was washed with brine. The organic layer was dried with Na2SO4, and the solvent was removed by evaporation. The crude product was purified by preparative HPLC to give 10 a–l. See Supporting Information for p-10b, p-10c, p-10d, p-10e, p-10f, p-10g, p-10h, p-10i, p-10j, m-10b, m-10c, m-10d, m-10e, m-10f, m-10g, o-10b, o-10c, o-10d, o-10e, o-10f, and o-10g.

Compounds 11a–b were prepared by following the procedure described for the synthesis of 8 (Supporting Information).

Representative Amidation. A solution of compound 7 (1.75 g, 7.2 mmol) in SOCl2 (3 mL) was heated to reflux for 2 h and then cooled to rt and concentrated under vacuum to give a pale-white solid. The crude acid chloride (1.87 g, 6.7 mmol) was dissolved in CH2Cl2 (25 mL) and was added to a stirred solution of the amine (6.7 mmol) and triethylamine (15.4 mmol) in CH2Cl2 at 0 °C, and the mixture was stirred for 4 h at rt, diluted with H2O and extracted with CH2Cl2 (2 × 30 mL) and concentrated under vacuum to provide 12a–c. Compounds were submitted to nitro reduction conditions as described for the synthesis of 9.

Compounds 13a–c were prepared by following the procedure described for the synthesis of 9 (Supporting Information).

Compounds 15a–c were prepared from 13 and 5 by following the procedure described for the synthesis of 10 (Supporting Information).

Representative Acylation and Fries Rearrangement. A mixture of 3-aminophenol (1.09 g, 10 mmol) and butyl anhydride (4.1 mL, 25 mmol) in pyridine (10 mL) was stirred at rt for 1.5 h. The reaction mixture was diluted with EtOAc (60 mL), and the organic layer was washed with sat. NaHCO3 (2 × 60 mL, 10% HCl (2 × 60 mL), dried (Na2SO4), and concentrated, and to the resulting oil was added 1.5 mL of 1,2-dichlorobenzene and AlCl3 (2.67 g, 20 mmol) and the solution was stirred at 120 °C overnight under nitrogen. The resulting tar was added to EtOAc (60 mL) and washed with 30 mL of water. The mixture was heated to 90 °C, and KMnO4 (14.2 g, 90 mmol) was added in portions. The mixture was refluxed for 5 h. The black solid was filtered off, and the filtrate was acidified with 6 N HCl. The mixture was cooled in an ice bath and the white precipitate was collected (7.3 g, 85%); mp 175–176 °C. 1H NMR CDCl3 δ 8.25–8.33 (m, 2H, aryl), 8.08 (d, J = 8.9 Hz, 1H, aryl), 7.41–7.51 (m, 3H, aryl), 7.31–7.39 (m, 2H, aryl). MS m/z 243.2 (M + H)+.

5-Nitro-biphenyl-2-carboxylic Acid (7). A solution of compound 7 (2.84 g, 11.7 mmol) in methanol (35 mL) was treated with SOCl2 (4.16 g, 35 mmol) dropwise at 0 °C. The mixture was heated to reflux for 6 h and then cooled to rt. Under nitrogen, spontaneous crystallization occurred. The residual solvent was then filtered off, and the resulting solid was left to dry overnight to give 8 (2.28 g, 76%). 1H NMR CDCl3 δ 8.25 (m, 2H), 7.92 (d, J = 8.4 Hz, 1H), 7.43 (m, 3H), 7.32 (dd, 2H), 3.67 (s, 3H, COOCH3). MS m/z 258.1 (M + H)+.

5-Amino-biphenyl-2-carboxylic Acid Methyl Ester (9). A mixture of 5-nitro-biphenyl-2-carboxylic acid methyl ester 8 (2.0 g, 7.8 mmol) and SnCl2·2H2O (8.8 g, 39 mmol) in EtOAc (75 mL) was stirred at reflux under nitrogen for 2.5 h. Upon cooling, saturated NaHCO3 (150 mL) was added. The organic layer was removed, and the aqueous layer was washed with EtOAc (2 × 100 mL). The combined organic layers were dried (Na2SO4) and concentrated to dryness to give 9 as a white solid (1.53 g, 86%). 1H NMR CDCl3 δ 7.80 (d, J = 8.4 Hz, 1H), 7.27–7.37 (m, 5H, aryl), 6.63 (dd, J = 8.4, 2.1 Hz, 1H), 6.56 (d, J = 8.4 Hz, 1H), 3.59 (s, 3H, COOCH3). MS m/z 228.3 (M + H)+.
The filtrate was diluted with toluene and washed with 10% citric acid solution to yield a red solid (6.94 g, 71%) after recrystallization from methanol to yield a red solid (6.94 g, 71%). The organic layers were combined, dried (Na2SO4), and concentrated under vacuum to get 18a-c as brown solid (365 mg, 96%) (Supporting Information).

Representative Suzuki Cross Coupling. To a mixture of 18 (193 mg, 0.83 mmol) and Pd(PPh3)4 (10 mol %) in DMF (5 mL) at 100 °C was added 3 M H2SO4 (11.5 mL, 35 mmol) and 1 M NaHCO3 (2 mL). The solution was stirred at reflux overnight under nitrogen. The sodium sulfate was removed, and the resulting solid was washed with water (30 mL) and dried with Na2SO4. Evaporation of organics resulted in a green solid, which was recrystallized from EtOAc to yield a white solid (81 mg, 54%). See Supporting Information for 19a-c.

Representative N-Acyl Deprotection and Reductive Amination. Biphenyl 19 (180 mg, 0.58 mmol) was dissolved in 5 mL i-PrOH, and NaOH (232 mg, 5.8 mmol in 0.5 mL H2O) was added. The solution was heated to reflux overnight, after which the solvent was removed under vacuum. The resulting solid was taken up into CH2Cl2 (30 mL) and washed with brine (30 mL). The solvent was removed by evaporation, and the resulting black solid was purified by column chromatography using a gradient of 1:1 Hex:EtOAc to yield a white solid (81 mg, 54%). See Supporting Information for 20a-c.

2-Phenyl-1-nitro-3-bromobenzene (22a). Sodium nitrite (2.66 g, 0.1 mmol), and potassium acetate (294 mg, 3.0 mmol, 1.5 equiv) in 3 M sulfuric acid (11.5 mL, 35 mmol) and then stirred for 1 h. To the resultant mixture, water (20 mL) was added followed by the addition ofaq KOH to raise the pH to about 9–10. The organic phase was separated and the aqueous phase was extracted with ether (2 × 100 mL). The combined organic layers were washed with saturated NaCl and dried over sodium sulfate. The ether layer was evaporated to dryness, and the crude material was purified by flash chromatography eluting at 10% EtOAc to afford 22f, 0.45 g (80%).

Synthesis of 23a-g. Compounds were prepared as described for compound 9.

Synthesis of 24b-f. Compounds were prepared as described for compound 10. See Supporting Information for 24b-g.

Representative Heterocyclic Heck Reaction: 3-(5-Nitro-biphenyl-2-yl)-benzoxazole (25f). A mixture of 2-phenyl-4-nitrobromobenzene (22a, 155 mg, 2.0 mmol), Pd(PPh3)4 (116 mg, 10 mol %) in DMF (5 mL) was placed under high vacuum for 15 min. The solution was purged with Ar for 15 min. While purging was continued, ZnCN2 (101 mg, 0.86 mmol) and formaldehyde (9.3 mmol) in THF (25 mL) was added dropwise at 0 °C. The resulting mixture was stirred for 20 min at 10 °C and acetic acid (22 mL) was added dropwise. The mixture was heated at 100 °C for 1 h, then TsOH·H2O (7.2 g, 38.0 mmol) was added and the reaction mixture was stirred at reflux. After 12 h, the reaction was cooled, extracted with CH2Cl2 (2 × 100 mL). The combined organic layers were washed with saturated NaHCO3 (2 × 50 mL) and brine (100 mL) and dried over Na2SO4. Evaporation of organics resulted in a green solid, which was recrystallized from EtOAc to obtain a white solid (1.7 g, 70%). 1H NMR (CDCl3) δ 8.30-8.35 (m, 2H), 8.28 (d, J = 2 Hz), 8.07 (d, J = 7 Hz), 7.72 (d, J = 9 Hz), 7.33-7.49 (m, 7H).

Synthesis of Compounds 26a-g. Compounds were prepared from 25a-c as described for the synthesis of 9.

Synthesis of Compounds 27a-g. Reductive amination with 26 and 5 was performed as for compound 10. See Supporting Information for 27a-g.

Synthesis of Compounds 32a-h. Preparation of compounds 32a-h were performed as described for 10. See Supporting Information for 32a-h.
Preparation of Compounds 35a–n. Compounds were prepared from 34a–n and 5 under reductive amination conditions as performed previously. See Supporting Information for 35a–k.

3,4-Diphenylnitrobenzene (37). The synthesis was carried out according to the procedure for 19. 1H NMR (acetone-d6): δ 7.58 (dd, 1H, J = 3 and 9 Hz), 7.51 (1H, J = 3 Hz), 7.00 (d, 1H, J = 9 Hz), 6.54–6.58 (m, 5H), 6.48–6.51 (m, 4H).

3,4-Diphenylalanine (38). The synthesis was carried out using 37 according to the procedure described for 9. 1H NMR (acetone-d6): δ 7.02–7.20 (m, 11H), 7.71–7.75 (m, 2H).

(3-Biphenyl-4-ylmethyl)-3H-imidazol-4-yl)methyl)[1,1′,2′,1″terphenyl]-4-ylamine (39). The reaction was carried out using 38 and 5 according to the procedure described for 10. Yellow solid, 40%; mp 195–199 °C. 1H NMR (acetone-d6): δ 7.68 (1H, S), 7.59–7.62 (m, 4H), 7.44 (t, 2H, J = 7.75 Hz), 7.35 (tt, 2H, J = 2 and 8 Hz), 7.26 (d, 2H, J = 8 Hz), 7.12–7.17 (m, 5H), 7.06–7.12 (m, 4H), 7.01–7.04 (m, 2H), 6.98 (s, 1H), 6.72 (dd, 1H, J = 2 and 8 Hz), 6.64 (m, 1H), 5.40 (s, 2H), 4.31 (s, 2H). 13C NMR (CDCl3): δ 146.65, 141.79, 141.47, 141.20, 138.87, 135.03, 131.58, 129.86, 129.71, 129.25, 128.85, 128.72, 127.81, 127.59, 127.24, 127.03, 126.45, 125.74, 115.19, 112.34, 46.88, 38.29. HRMS [FAB M + H]+: C33H28N3 calcd, 492.2439; found, 492.2440.

4-Methyl-3-nitro-biphenyl (40). A large scale synthesis of 40 was carried out as follows. A mixture of 4-bromo-2-nitrotoluene (1.0 g, 4.6 mmol), phenyl boronic acid (0.6 g, 5.09 mmol), and Ba(OH)2·8H2O (3.2 g, 9.2 mmol) in 15 mL of DME/H2O (5:1) was stirred under Ar for 15 min. To this, Pd(PPh3)4 (0.53 g, 0.46 mmol) was added and the resulting solution was refluxed for overnight. The reaction was cooled and diluted with EtOAc (30 mL) and washed with NaHCO3 and brine. The resulting organic layer was filtered through celite, dried over Na2SO4, and filtered. The resulting paste was triturated with hexane to obtain a white solid (0.89 g, 90%). 1H NMR (CDCl3): δ 7.80 (1H), 7.69–7.76 (m, 2H), 7.44 (m, 2H), 7.24 (m, 2H), 6.99 (s, 1H), 6.72 (dd, 1H, J = 2 and 8 Hz), 6.64 (m, 1H), 5.40 (s, 2H), 4.31 (s, 2H). 13C NMR (CDCl3): δ 146.65, 141.79, 141.47, 141.20, 138.87, 135.03, 131.58, 129.86, 129.71, 129.25, 128.85, 128.72, 127.81, 127.59, 127.24, 127.03, 126.45, 125.74, 115.19, 112.34, 46.88, 38.29. HRMS [FAB M + H]+: C33H28N3 calcd, 492.2439; found, 492.2440.

Preparation of Posaconazole. See Supporting Information for 43b, 43c, and 43f.

Benzothiazol-2-yl-biphenyl-3-yl)-[3-(3-nitro-biphenyl-4-ylmethyl)-3H-imidazol-4-yl)methyl](43d). A large scale synthesis based on previous work25 was carried out as follows. To a solution of 26d (1.1 g, 3.67 mmol) and 42 (1.12 g, 3.67 mmol) in CH2Cl2 (40 mL) was added TiCl3 (1 M solution in dichloromethane) (1.83 mL, 1.83 mmol) dropwise at rt, and the mixture was stirred for 30 min. To this was added NaCNBH3 (276 mg, 4.4 mmol) in MeOH (6 mL), the resulting mixture was stirred overnight, and the solvents was removed by evaporation. The resulting crude material was dissolved in CH2Cl2 (150 mL) and sat. NaHCO3 (50 mL). The organic layer was separated and dried (Na2SO4) to obtain a yellow solid, which was recrystallized from EtOH to afford 43d (1.5 g, 70%). 1H NMR (CD3OD): δ 7.80 (1H), 7.69–7.76 (m, 2H), 7.44–7.39 (m, 2H), 2.65 (s, 3H). MS m/z: 214.2 (M + H)+.

Preparation of Compounds 44a–e. Compounds were synthesized by following the procedure described for 9.

Preparation of T. cruzi and Murine Fibroblast Growth Inhibition Assays. Compounds were screened against the β-galactosidase expressing the Tulahuen strain of T. cruzi in 96-well tissue culture plates as described previously.25,26 The Tulahuen strain originated in Chile and is grouped with the more common TC1 phylogenetic lineage of T. Cruzii. In this assay, T. cruzi proliferates as intracellular amastigotes within murine 3T3 fibroblasts. Compounds were screened in triplicate to determined values of EC50. Standard errors within assays were consistently less than 4 μmol.

Inhibitors of Lanosterol 14α-Demethylase in Chagas Disease

than 15%. Compounds were separately screened against murine T3T fibroblast cells to determine the EC\textsubscript{50} values against these host cells. Growth of the T3T fibroblasts was quantified by the resazurin assay as previously described.\textsuperscript{25} Although some compounds were yellow-colored as solids, there was no effect of the diluted compounds on the colorimetric readouts.

**Pharmacokinetic Studies in Mice.** Compounds were suspended at 10 mg/mL in 20\% (w/v) Trappsol hydroxypropyl β-cyclodextrin (pharmaceutical grade) (CTD, Inc.) and administered to BALB/c mice (7–8 week females weighing approximately 20 g) by oral gavage in a volume of 100 μL. Thus, the mice received a dose of 50 mg/kg. At timed intervals, 40 μL of tail blood was collected in heparinized capillary tubes. Plasma was separated and frozen for later analysis according to the method previously reported.\textsuperscript{27} Individual traces of plasma drug concentration versus time for each mouse are provided as Supporting Information.

**Efficacy Studies in mice.** BALB/c mice (7–8 week females) were infected with 1 × 10\textsuperscript{4} T. cruzi trypomastigotes (Tulahuen strain) by subcutaneous injection. By 7 days postinfection, every mouse had microscopically observable parasites on slides of peripheral blood. On day 7 postinfection, mice (in groups of 6) began receiving treatments by oral gavage twice per day for 21 days. Compounds were administered in a volume of 100 μL per dose using the vehicle, 20\% (w/v) Trappsol hydroxypropyl β-cyclodextrin (pharmaceutical grade) (CTD, Inc.). Parasitemia was monitored by placing 5 μL of tail blood under a coverslip and counting 50 high-powered fields. Mice that were precordial from progressive infection were euthanized. All surviving mice were sacrificed on day 100 postinfection, and ~200 μL of blood from cardiac puncture was taken for PCR analysis of parasitemia.

**L14DM Binding Studies.** Expression of T. cruzi L14DM in E. coli and purification would be reported elsewhere. Binding reactions contained 2.2 μM T. cruzi L14DM (concentration determined from the Soret peak, absorbance at 420 nm minus absorbance at 490 nm using an extinction coefficient of 111 mm\textsuperscript{-1} cm\textsuperscript{-1}) in 1 mL of 50 mM sodium phosphate, pH 7.5, 300 mM NaCl, 10% glycerol. Binding of inhibitors was monitored by difference spectroscopy (340 nm) in which 1 mL of the above protein solution was placed in the sample and reference cuvettes. After setting the difference spectrum to zero, 1 μL aliquots of inhibitor stock solution (200 μM in DMSO) was added to the sample compartment, and 1 μL of DMSO only was added to the reference cuvette. The inhibitor concentration was varied from 200 to 2000 nM.

**PCR Detection of T. cruzi Parasitemia.** Extraction of T. cruzi DNA and PCR amplification of DNA was performed according to previously published methods.\textsuperscript{28–30} Briefly, 200 μL of the whole was mixed with 200 μL of 6 M guanidine HCl–0.2 M EDTA and stored at 4 °C. Samples were boiled for 15 min and extracted with an equal volume of phenol:chloroform (1:1) and then extracted with an equal volume of chloroform:isoamyl alcohol (24:1), followed by ethanol precipitation and resuspension in 20 μL of H\textsubscript{2}O. Precipitated DNA (2 μL of a 1:20 dilution) was subjected to PCR in a volume of 50 μL for 39 cycles using the S35 and S36 primers that amplify a 330-bp minicircle sequence.\textsuperscript{30} Then 6 μL of the PCR products were separated on 2% agarose gel and visualized with ethidium bromide.

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**Supporting Information Available:** Pharmacokinetic data for compounds 44a and 44d and mice weight data. This material is available free of charge via the Internet at http://pubs.acs.org.

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