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2,4-Diamo-6-methylpyrimidines for the potential treatment of Chagas’ Disease

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Abstract: Chagas’ disease, caused by the protozoan parasite Trypanosoma cruzi, affects 8-10 million people across the Latin American population and is responsible for around 12,500 deaths per annum. The current frontline treatments, benznidazole and nifurtimox, are associated with side effects and lack efficacy in the chronic stage of the disease, leading to an urgent need for new treatments. A high throughput screening campaign against the physiologically relevant intracellular form of the parasite identified a series of 2,4-diamino-6-methylpyrimidines. Demonstrating the series did not work through the anti-target TcCYP51, and was generally cytocidal, confirmed its suitability for further development. This study reports the optimisation of selectivity and metabolic stability of the series and identification a suitable lead for further optimisation.
Chagas’ Disease\(^1\), an infection caused by the parasite *Trypanosoma cruzi*, is endemic in 21 countries across Latin America and is estimated to be responsible for around 12,500 deaths per Annum\(^2\). The disease is also found in many other parts of the world, mainly due to migration. Chagas’ disease is characterized by an initial acute phase, which is fatal for around 5% of children. This is followed by a long-term chronic phase, which can eventually be fatal due to associated cardiac problems\(^3,4\). The standard treatment is based on benznidazole and nifurtimox, nitro-aromatic drugs that effectively clear parasites in the acute phase, but are less effective in the chronic phase\(^5\). There are no current alternatives to benznidazole or nifurtimox and recent clinical trials of posaconazole, an inhibitor of the enzyme *T. cruzi* C14 \(\alpha\)-demethylase (commonly known as TcCYP51) did not give promising results\(^6\). Thus, there is an urgent need for novel chemical classes that target *T. cruzi* through alternative mechanisms to TcCYP51.

To this end, we have developed a screening cascade to identify chemical series with the potential to treat Chagas’ disease (see fig. 1)\(^7\). An initial high throughput phenotypic screen against the intracellular amastigote form of *T. cruzi* identified compounds that inhibit parasite growth with selectivity over host Vero cells\(^7\). These compounds were then counter-screened against TcCYP51 to identify those acting via alternative, non-TcCYP51 mechanisms\(^8\). Alongside this, a second *T. cruzi* assay identified compounds acting through a cytocidal, rather than cytostatic mode of action\(^7\). Series thus identified could be further optimized to give compounds suitable for progression into an in vivo efficacy model of Chagas’ disease.

---

**Diagram:**

1. Single point library screen
2. Intracellular amastigote assay
3. 10-Point potency
4. Intracellular amastigote assay
5. Potency / selectivity cut-off
6. 10-point cytostatic/cytocidal assay
7. TcCYP51 Assay
Figure 1: Screening cascade to identify chemical series for development against *T. cruzi*

A set of >13,000 compounds with antimalarial activity was released by GSK (referred to as TCAMS)\(^9\), and this set was screened through the cascade described. One of the series identified from this screen was exemplified by compound 1 (Table 1), which demonstrated a \(\text{pEC}_{50}\) against *T. cruzi* parasites of 6.9 and a 40-fold selectivity window versus host Vero cells. Compound 1 was further profiled through the highlighted cascade, showing cytocidal behavior and no \(Tc\)CYP51 activity (i.e. \(\text{pEC}_{50}\) <5). In terms of *in vitro* DMPK properties, the compound had low kinetic solubility but was stable in mouse liver microsomes (MLM). Further examination of the hit set identified a number of close analogues that all contained the 1,4-dianilino moiety, with either amide or sulfonamide linkers, which further validated this series. These analogues all had a relatively similar selectivity window versus the host cells (40-60 fold) and poor solubility (data not shown). This programme therefore focused on optimizing potency, selectivity and solubility to identify compounds suitable for progression into an *in vivo* model of Chagas’ disease.

Table 1. Hit Compound 1.

<table>
<thead>
<tr>
<th>No.</th>
<th>(Tc) (\text{pEC}_{50})^(a)</th>
<th>Vero (\text{pEC}_{50})^(a)</th>
<th>Selectivity^b</th>
<th>Solubility µM^c</th>
<th>(\text{Cl}^{\text{int}}) (MLM) ml/min/g^d</th>
<th>(Tc)CYP51 pIC(_{50})</th>
<th>Cytocidal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.8±0.2</td>
<td>5.2±0.3</td>
<td>40</td>
<td>20</td>
<td>0.7</td>
<td>&lt;5</td>
<td>Yes</td>
</tr>
</tbody>
</table>

^a\(Tc\) refers to activity in *T. cruzi* amastigote cultures in Vero cells. \(\text{pEC}_{50}\) value shown as mean values from three \(\text{EC}_{50}\) determinations ± standard deviation. ^b Selectivity refers to Vero \(\text{pEC}_{50}/Tc\) \(\text{pEC}_{50}\). ^c Kinetic solubility measured in water from a DMSO solution of compound. ^d \(\text{Cl}^{\text{int}}\) (MLM) refers to intrinsic clearance measured in mouse liver microsomes.
This 2,4-diamino-6-methylpyrimidine motif has previously been reported to show antimalarial activity\textsuperscript{10} and has more recently been identified in screens against the kinetoplastids\textsuperscript{11}. As far as we are aware, this is the first report of developing this compound series for the treatment of Chagas' disease.

Chemistry to access the desired analogues is described in Schemes 1-3. Compounds with variations in the 2-pyrimidinyl position were synthesized according to Scheme 1\textsuperscript{10}. Commercially available 2,4-dichloro-6-methylpyrimidine 2 was treated with dimethylamine, followed by a suitably substituted aniline to give compounds 4a-d, whilst treatment with \( m \)-or \( p \)-aminobenzoate, followed by hydrolysis of the ester, gave acids that were treated with TBTU and a relevant amine to give amides 5a-c. Compound 3 could also be reacted with alkyl amines under basic conditions to give compounds 6a-e, whilst treatment with 1,4-trans-cyclohexanediamine and subsequent acylation gave 8a-b. Compounds 18a-e could be synthesized following an identical route, starting from suitably functionalized 2,4-dichloropyrimidines.

Analogues with variations to the pyrimidinyl 4-position were synthesised according to Scheme 2. The thiopyrimidine 9 was alkylated with iodomethane and the resulting thioether

\begin{center}
\includegraphics[width=0.8\textwidth]{scheme1.png}
\end{center}

\textbf{Scheme 1.} Synthesis of 4a-d, 5a-c, 6a-e and 8a-b. (a) Me\(_2\)NH, THF, 68%; (b) Aniline, Pd(\( \text{Bu}_3\)P), \( \text{NaO}^+\text{Bu} \), 1,4-dioxane, 120°C, 21-68%; (c) 1M NaOH, THF then TBTU, DIPEA, DMF; (d) 1,4-dioxane, 160°C; (e) R\(_4\)-COCl, DIPEA, DCM.
displaced by 3-methoxy-4-fluoro aniline to give 11. This was treated with POCl₃ and the resulting chloro-group displaced by relevant amines to give compounds 13a-g.

![Scheme 2. Synthesis of 13a-g.](image)

**Scheme 2.** Synthesis of 13a-g. (a) Iodomethane, potassium carbonate, DMSO, 48%; (b) Aniline, butan-1-ol, 110°C, 49%; (c) POCl₃, 120°C, 59%; (d) R₁R₂-NH₂, DIPEA, 1,4-dioxane, 110°C

In order to access 2-benzylamino-4-anilino compounds, 2,4-dichloro-6-methyl pyrimidine 14 was treated with benzylamine, giving a mixture of the 2- and 4- substituted intermediates (15 and 16), which were easily separated and identified by NMR (Scheme 3). Compound 16 was treated with 3-methoxy-4-fluoroaniline under acidic conditions to give 17a-c.

![Scheme 3. Synthesis of 17a-c.](image)

**Scheme 3.** Synthesis of 17a-c. (a) Benzylamine, DIPEA, THF (b) relevant aniline, conc. HCl, EtOH, 50%.

The focus of the initial medicinal chemistry program was to improve solubility and selectivity, targeting a pEC₅₀ > 7 and cellular selectivity of > 100-fold in the intracellular amastigote assay⁷, solubility of > 250 μM and Clₕ < 5 ml/min/g. These parameters were based on previous experience of progressing compound series into in vivo models of Chagas’ disease¹². Since this series was identified through a phenotypic screen, the molecular target
was unknown; therefore we undertook a systematic exploration of the pyrimidine substituents to address the progression parameters.

An initial strategy was to remove / replace the 1,4-dianilino moiety, which we believed could be a key driver of the poor solubility of 1, as well as presenting potential genotoxicity risks as the compound series progressed. Also, removal of the benzamide would reduce the number of aromatic rings, which could also help to improve solubility. The initial round of synthesis investigated whether either aminobenzamides or other substituted anilines would result in improved profiles (Table 2). The para-substituted piperidine amide 5a showed no toxicity against the host cells at the top concentration tested (50 µM), but had reduced potency compared to compound 1. Phenyl and benzyl amides, as exemplified by 5b and 5c, had a similar potency but reduced selectivity compared to 5a. Further exploration of the benzamides, including meta-substituted benzamides, failed to improve potency.

**Table 2: In vitro profile of analogues 5a-c, 4a-d and 6a-c.**

<table>
<thead>
<tr>
<th>No.</th>
<th>R</th>
<th>Tc pEC(_{50})</th>
<th>Vero pEC(_{50})</th>
<th>Selectivity</th>
<th>Solubility µM</th>
<th>Cl(_{int}) (MLM) ml/min/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>6.8±0.2</td>
<td>5.2±0.3</td>
<td>40</td>
<td>20</td>
<td>0.7</td>
</tr>
<tr>
<td>5a</td>
<td>( \text{para} )</td>
<td>5.7±0.3</td>
<td>&lt;4.3</td>
<td>&gt;25</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>5b</td>
<td>( \text{para} )</td>
<td>6.1±0.2</td>
<td>6.2±0.4</td>
<td>1.3</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>5c</td>
<td>( \text{para} )</td>
<td>5.9±0.4</td>
<td>5.3±0.3</td>
<td>4</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>4a</td>
<td>( \text{para} )</td>
<td>5.8±0.3</td>
<td>&lt;4.3</td>
<td>&gt;32</td>
<td>&gt;250</td>
<td>10</td>
</tr>
<tr>
<td>4b</td>
<td>( \text{para} )</td>
<td>6.1±0.4</td>
<td>&lt;4.3</td>
<td>&gt;63</td>
<td>&gt;250</td>
<td>15</td>
</tr>
<tr>
<td>Compound</td>
<td>Tc EC&lt;sub&gt;50&lt;/sub&gt; (μM)</td>
<td>Vero EC&lt;sub&gt;50&lt;/sub&gt; (μM)</td>
<td>Selectivity</td>
<td>Solubility</td>
<td>Cl&lt;sub&gt;int&lt;/sub&gt; (MLM)</td>
<td>Notes</td>
</tr>
<tr>
<td>----------</td>
<td>----------------</td>
<td>-----------------</td>
<td>-------------</td>
<td>-----------</td>
<td>----------------</td>
<td>------</td>
</tr>
<tr>
<td>4c</td>
<td>5.9±0.3</td>
<td>&lt;4.3</td>
<td>&gt;40</td>
<td>&gt;250</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>4d</td>
<td>6.4±0.1</td>
<td>4.5±0.1</td>
<td>80</td>
<td>&gt;250</td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>6a</td>
<td>5.5±0.3</td>
<td>&lt;4.3</td>
<td>&gt;16</td>
<td>&gt;250</td>
<td></td>
<td>28</td>
</tr>
<tr>
<td>6b</td>
<td>5.9±0.1</td>
<td>&lt;4.3</td>
<td>&gt;40</td>
<td>&gt;250</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>6c</td>
<td>6.0±0.3</td>
<td>4.6±0.2</td>
<td>25</td>
<td>&gt;250</td>
<td></td>
<td>12</td>
</tr>
</tbody>
</table>

<sup>a</sup> Tc refers to activity in *T. cruzi* amastigotes cultures in Vero cells. pEC<sub>50</sub> values are shown as mean values from two EC<sub>50</sub> determinations ± range (except 1, 4b where value is ± standard deviation from >2 determinations).  
<sup>b</sup> Selectivity refers to Vero pEC<sub>50</sub>/Tc pEC<sub>50</sub>.  
<sup>c</sup> Kinetic solubility measured in water from a DMSO solution of compound.  
<sup>d</sup> Cl<sub>int</sub> (MLM) refers to intrinsic clearance measured in mouse liver microsomes. N/A means value was not measured.

Because the strategy of replacing the anilide with a benzamide had not led to improved compounds, we investigated a wider range of substituted anilines (exemplified by 4a-d in Table 2). Pleasingly, both m- and p-methoxy analogues (4a and 4b) showed similar potencies to compounds 5a-c with an improved selectivity window and solubility compared to 1. Alongside potency, microsomal clearance for 4a and 4b required further optimization; hence we synthesized further substituted anilines and benzylamines to address these issues. All attempts, as exemplified by 4c, 4d, and 6a-c led to analogues with similar potency and clearance values.

As we had made a significant change to the original hit scaffold, we confirmed that compounds 4d and 6a did not inhibit TcCYP51 and both were cytocidal. Alongside that, profiling of 4d in a panel of potential off target receptors associated with *in vivo* toxicity, revealed that inhibition of hERG (pIC<sub>50</sub> 5.3) was a potential issue, due to its association with potentially fatal arrhythmia. This data did not stop the progression of the series, but would need addressing if the compound series were ultimately to be developed.
Encouraged by 4d and 6a, which were highly selective and soluble, further SAR was explored around these scaffolds to improve metabolic stability (See Tables 3 and 4) by removing the potential for N-demethylation of the 4-dimethylamino group. Analogues of 4d, represented by 13a-g (Table 3), where the dimethylamine was replaced with other alkyl amines, failed to significantly improve metabolic stability (where Cl_{int}<5ml/min/g was targeted). Additionally, none of the changes, which introduced both polar and non-polar groups, led to a significant improvement in potency.

**Table 3**: In vitro profile of analogues 13a-g.

<table>
<thead>
<tr>
<th>No.</th>
<th>R</th>
<th>Tc pEC_{50}</th>
<th>Vero pEC_{50}</th>
<th>Selectivity</th>
<th>Solubility (μM)</th>
<th>Cl_{int} (MLM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4d</td>
<td></td>
<td>6.4±0.3</td>
<td>4.5±0.1</td>
<td>80</td>
<td>&gt;250</td>
<td>17</td>
</tr>
<tr>
<td>13a</td>
<td></td>
<td>6.0±0.4</td>
<td>4.8±0.2</td>
<td>16</td>
<td>160</td>
<td>17</td>
</tr>
<tr>
<td>13b</td>
<td></td>
<td>6.5±0.5</td>
<td>4.7±0.2</td>
<td>63</td>
<td>220</td>
<td>14</td>
</tr>
<tr>
<td>13c</td>
<td></td>
<td>5.1±0.4</td>
<td>&lt;4.3</td>
<td>&gt;6</td>
<td>&gt;250</td>
<td>18</td>
</tr>
<tr>
<td>13d</td>
<td></td>
<td>5.8±0.3</td>
<td>4.6±0.2</td>
<td>16</td>
<td>&gt;250</td>
<td>35</td>
</tr>
<tr>
<td>13e</td>
<td></td>
<td>5.1±0.2</td>
<td>4.4±0.1</td>
<td>5</td>
<td>219</td>
<td>N/A</td>
</tr>
<tr>
<td>13f</td>
<td></td>
<td>5.1±0.2</td>
<td>4.5±0.2</td>
<td>4</td>
<td>&gt;250</td>
<td>35</td>
</tr>
<tr>
<td>13g</td>
<td></td>
<td>6.1±0.5</td>
<td>5.3±0.2</td>
<td>6.3</td>
<td>&gt;250</td>
<td>8</td>
</tr>
</tbody>
</table>
a Tc refers to activity in T. cruzi amastigote cultures in Vero cells. pEC$_{50}$ value shown as mean values from three EC$_{50}$ determinations ± standard deviation. b Selectivity refers to Vero pEC$_{50}$/Tc pEC$_{50}$. c Kinetic solubility measured in water from a DMSO solution of compound. d Cl$_{int}$ (MLM) refers to intrinsic clearance measured in mouse liver microsomes.

To further explore replacement of the 4-dimethylamino group, we focused on the introduction of substituted anilines. Due to concern that addition of a further aromatic ring could reduce solubility, these changes were introduced onto the benzyl substituted scaffold of 6a, rather than the aniline 4d, where the increased sp$^3$ character might alleviate this potential issue\textsuperscript{13}. As shown in Table 4, these analogues (17a-c) demonstrated improved potency whilst maintaining selectivity and solubility compared to compound 6a, although none of them improved the metabolic stability. 17c did show a significant improvement in potency compared to 6a, achieving our target of pEC$_{50} > 7.0$, and also contained significant structural changes. Because of this, 17c was further profiled in our cytostatic / cytocidal assay where it demonstrated cytostatic behavior, suggestive of a change in the mode of action of this compound. This was undesirable, because our previous experience of a cytostatic compound was that it would not achieve cures in in vivo models\textsuperscript{12}. As such, this sub-series was not progressed further.

Table 4: In vitro profile of analogues 17a-c.

<table>
<thead>
<tr>
<th>No.</th>
<th>R</th>
<th>Tc pEC$_{50}$</th>
<th>Vero pEC$_{50}$</th>
<th>Selectivity b</th>
<th>Solubility µM c</th>
<th>Cl$_{int}$ (MLM) ml/min/g d</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a</td>
<td>H</td>
<td>5.5±0.3</td>
<td>&lt;4.3</td>
<td>&gt;16</td>
<td>&gt;250</td>
<td>28</td>
</tr>
<tr>
<td>17a</td>
<td>F</td>
<td>6.4±0.4</td>
<td>5.1</td>
<td>20</td>
<td>&gt;250</td>
<td>10</td>
</tr>
<tr>
<td>17b</td>
<td>Cl</td>
<td>6.7±0.1</td>
<td>5.1±0.1</td>
<td>40</td>
<td>&gt;250</td>
<td>20</td>
</tr>
</tbody>
</table>
Returning to the 2-(3-methoxy-4-fluoroanilino)-4-dimethylamino template of 4d, which retained the same *in vitro* profile as compound 1, we investigated variations to the 6-methyl group (Table 5), focusing on changes which could enhance metabolic stability. As can be seen from Table 5, all analogues investigated demonstrated a significant reduction in potency although 18e was the first compound in the series to demonstrate progressable metabolic stability.

**Table 5**: In vitro profile of analogues 18a-e.

<table>
<thead>
<tr>
<th>No.</th>
<th>R</th>
<th>Tc pEC₅₀ ²</th>
<th>Vero pEC₅₀ ²</th>
<th>Selectivity ³</th>
<th>Solubility</th>
<th>Cl₉₀ (MLM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µM ³⁶</td>
<td>ml/min/g ³⁶</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4d</td>
<td>H⁺</td>
<td>6.4±0.3</td>
<td>&gt;250</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18a</td>
<td>H⁺</td>
<td>5.4±0.1</td>
<td>&lt;4.3</td>
<td>160</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>18b</td>
<td>H⁺</td>
<td>5.1±0.1</td>
<td>&lt;4.3</td>
<td>25</td>
<td>&gt;250</td>
<td>35</td>
</tr>
<tr>
<td>18c</td>
<td>(CH₃)₂</td>
<td>5.7±0.3</td>
<td>&lt;4.3</td>
<td>170</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>18d</td>
<td>(CF₃)₂</td>
<td>4.6±0.5</td>
<td>&lt;4.3</td>
<td>110</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>18e</td>
<td>(CO₂H)₂</td>
<td>4.5±0.2</td>
<td>&lt;4.3</td>
<td>&gt;250</td>
<td>3.4</td>
<td></td>
</tr>
</tbody>
</table>

² Tc refers to activity in *T. cruzi* amastigotes cultures in Vero cells. pEC₅₀ values are shown as mean values from three or more EC₅₀ determinations ± standard deviation. ³ Selectivity refers to Vero pEC₅₀/Tc pEC₅₀. ⁴ Kinetic solubility measured in water from a DMSO solution of compound. ⁵ Cl₉₀ (MLM) refers to intrinsic clearance measured in mouse liver microsomes. N/A means value was not measured.
As variations to the 4- and 6-positions of the pyrimidine had failed to identify compounds with suitable profiles to progress to in vivo studies, our focus returned to the pyrimidinyl 2-position. From previous SAR (outlined within Table 1 and 2) it was known that variations in this position could be tolerated without loss of potency. Also, 18e gave us optimism that analogues with improved metabolic stability could be identified within the series.

Table 6 shows a comparison of these analogues with 4d and 6a, where we were able to identify compounds with a similar level of potency, solubility and selectivity. Critically we observed a significant improvement in metabolic stability, most notably for compound 8b. This compound had a pEC\textsubscript{50} of 6.4 as well as very good selectivity (100-fold) and solubility, and no activity versus TcCYP51. Compound 8b was also cytotoxic, albeit with a narrow selectivity window; this lower selectivity likely being due to the two assays being run in different cell lines. Finally 8b was profiled against the same panel of off target receptors that was used for 4d, unfortunately showing no improvement. In fact, further risks were identified; hERG (pIC\textsubscript{50} 5.4), Aurora B (pIC\textsubscript{50} 7.4, highlighting potential promiscuity issues with other kinases) and CB1 (pIC\textsubscript{50} 7.2, concerns of psychotropic effects). These changes demonstrated that the cyclohexylidiamine linker gave a scaffold with the potential for further modifications to improve the profile against T. cruzi, but introduced potential toxicity risks which could hamper the development of this series.

Table 6: In vitro profile of analogues 4d, 6a, 6d-e and 8a-b.

<table>
<thead>
<tr>
<th>No.</th>
<th>( R )</th>
<th>Tc pEC\textsubscript{50} ( ^a )</th>
<th>Vero pEC\textsubscript{50} ( ^a )</th>
<th>Selectivity ( ^b )</th>
<th>Solubility ( ^c )</th>
<th>Cl\textsubscript{int} (MLM) ( ^d )</th>
</tr>
</thead>
<tbody>
<tr>
<td>4d</td>
<td></td>
<td>6.4±0.3</td>
<td>4.5±0.1</td>
<td>80</td>
<td>&gt;250</td>
<td>17</td>
</tr>
<tr>
<td>6a</td>
<td></td>
<td>5.5±0.3</td>
<td>&lt;4.3</td>
<td>&gt;16</td>
<td>&gt;250</td>
<td>28</td>
</tr>
<tr>
<td>6d</td>
<td></td>
<td>4.8±0.1</td>
<td>&lt;4.3</td>
<td>&gt;3</td>
<td>&gt;250</td>
<td>3.8</td>
</tr>
</tbody>
</table>
In summary, a novel series of *T. cruzi* inhibitors was identified through high throughput screening against the intracellular parasite that were demonstrated to be cytocidal, and also to be working via a non-*Tc*CYP51 mechanism. These initial hits suffered from poor selectivity and low solubility and microsomal stability. A series of compounds was therefore synthesized to address these concerns. The initial rounds of optimisation produced compound 17c, with acceptable potency and improved solubility and selectivity. However, 17c was no longer cytocidal, suggesting that its mode of action had changed, and also highlighting the need to use *Tc*CYP51 and cytocidality assays when working on a series where the mode of action was unknown. A different approach of using non-aromatic linkers to mimic the diaminino moiety of the initial hits led to 8b; this compound was moderately potent (pEC₅₀ 6.4), highly selective over the host Vero cells and displayed improved microsomal stability, albeit with increased off-target risks leading to the potential for *in vivo* toxicity. As the series was progressed phenotypically, it could potentially show polypharmacology, with different molecules having varying inhibition profiles, making optimisation challenging. Although further exploration of the scaffold could give compounds with suitable profiles for progression to *in vivo* studies, knowledge of the molecular targets and binding mode(s) of the series would facilitate this optimisation against targets which are cytocidal, and also offer strategies to design out off-target effects.
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References


Optimising selectivity and metabolic stability of anti-Chagas disease pyrimidines
Structure activity relationships development to optimise developability properties
Development of compound not targeting *T. cruzi* C14 α-demethylase
Improved selectivity and solubility

1

$\text{pEC}_{50} \text{ vs. } T. \text{ cruzi} = 6.8$
Selectivity vs. host cell = 40
Aqueous solubility $= 20 \mu M$

8b

$\text{pEC}_{50} \text{ vs. } T. \text{ cruzi} = 6.4$
Selectivity vs. host cell = 100
Aqueous solubility $> 250 \mu M$