pK$_a$ modulation of a bis(2-aminomimidazoline) DNA minor groove binder that targets the kinetoplast of *Trypanosoma brucei*

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pKₐ modulation of a bis(2-aminoimidazoline) DNA minor groove binder that targets the kinetoplast of *Trypanosoma brucei*

100 % curative in a mouse model of first stage HAT.
Abstract:

The parasite Trypanosoma brucei, ethiologic agent of human African trypanosomiasis (i.e. sleeping sickness), contains a kinetoplast with the mitochondrial DNA (kDNA) comprising of >70 % AT base pairs. Hence, DNA minor groove binding molecules have been investigated as antitrypanosomal agents. Diphenyl-based bis(2-iminoimidazolidines) are promising DNA minor groove binders that are curative in mouse models of stage 1 trypanosomiasis but devoid of activity in the late(CNS)-stage disease, possibly due to poor brain penetration caused by their dicationic nature.

As a strategy to reduce the pK\textsubscript{a} of the basic 2-iminoimidazolidine groups, halogen atoms (R\textsubscript{1} = Cl, F) were introduced in the structure of lead compound 1 [1] and the pK\textsubscript{a} of the new compounds was determined. A reduction of 1–2 pK\textsubscript{a} units for the imidazolidine group linked to the substituted phenyl ring was observed [1,2]. In vitro activities (EC\textsubscript{50}) against wild type and resistant strains of T. b. brucei were in the submicromolar range with four compounds being more active and selective than 1 (SI > 340). The chloro-substituted derivative 5a, which was curative in vivo in a mouse model of stage 1 infection by T. b. rhodesiense, appeared as a new promising lead compound.

Mechanistic studies were performed to identify the cellular target of these dicationic compounds. Altogether, our results show that 1 and 5a share the same mechanism of action against T. brucei, acting specifically on the integrity of the kinetoplast by altering the structure and replication of kDNA [3].

Keywords: DNA minor groove binder, imidazolidine, dicationic compound, Trypanosoma brucei, kinetoplast
Introduction

Neglected tropical diseases (NTDs)

Human African trypanosomiasis (HAT), also known as sleeping sickness, is a neglected tropical disease that is almost invariably fatal if left untreated.

It is caused by subspecies of the protozoan parasite Trypanosoma brucei which is transmitted to humans by the tsetse fly vector. Approximately 55 million people distributed over a surface of 340 000 km² in 33 sub-Saharan Africa countries are estimated to be at different levels of risk of contracting sleeping sickness¹.

DISEASE STAGES:

- Haemolymphatic stage (affects the blood)
- Brain stage (affects the CNS)

Most of the affected populations live in remote rural areas with limited access to health services, which complicates the diagnosis and treatment of cases in Africa's poorest countries.

Distribution of human African trypanosomiasis (T.b.gambiense), worldwide, 2016

Distribution of Human African Trypanosomiasis (T.B. rhodesiense), 2016
Date : 27/Sep/2017
Annual country reports, 2016

http://apps.who.int/neglected_diseases/ntddata/hat/hat.html Access: June 12th, 2018
http://gamapserver.who.int/mapLibrary/app/searchResults.aspx Access: June 12th, 2018
Chemotherapy

The current drugs used to treat HAT (suramin, pentamidine, melarsoprol and nifurtimox-eflornithine combination therapy, or NECT) are toxic and sometime ineffective due to the appearance of drug-resistant strains of *T. brucei*.\(^1\) In the last decades, efforts have been made to discover improved drugs to treat HAT.

Hit Identification

IC$_{50}$ = 0.025 µM* SI = >240

pK$_{a(1)}$ = 9.04 pK$_{a(2)}$ = 10.26

% ionization at pH 7.4 = 98.7*

100 % curative in a mouse model of first stage HAT. *

**Hit compound I (FR60)**

In previous reports, we have shown that compound 1, 4-((4,5-dihydro-1H-imidazol-2-yl)amino)-N-(4-((4,5-dihydro-1H-imidazol-2-yl)amino)phenyl)benzamide dihydrochloride, displayed excellent antitrypanosomal activity *in vitro* and were selective toward *Trypanosoma brucei*.

Compound 1 was curative by oral administration in a mouse model of acute *T. b. rhodesiense* infection demonstrating a great potential as chemotherapeutic agent.


Parasite target

The parasite *Trypanosoma brucei*, contains a kinetoplast with the mitochondrial DNA (kDNA) comprising of >70% AT base pairs.

Bisimidazoline compounds are DNA minor groove binding molecules that specifically target AT base pairs, so the kinetoplast is a likely target of our compounds.

We thus performed several experiments to understand the mode of action of these compounds.

1 Vargas-Parada, L. Kinetoplastids and their Networks of Interlocked DNA. *Nature Education, 2010*, 3(9):63
Synthetic strategy

## Results and discussion

### Physicochemical characterization: $pK_a$

<table>
<thead>
<tr>
<th>Index</th>
<th>$R^1$</th>
<th>$R^2$</th>
<th>$R^3$</th>
<th>$R^4$</th>
<th>UV-metric $pK_a$ (1)</th>
<th>UV-metric $pK_a$ (2)</th>
<th>pH-metric $pK_a$ (1)</th>
<th>pH-metric $pK_a$ (2)</th>
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<tbody>
<tr>
<td>1</td>
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<tr>
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<td></td>
<td></td>
<td>F</td>
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<td>8.30</td>
<td>9.70</td>
<td>8.29</td>
<td>9.98</td>
</tr>
</tbody>
</table>

**Influence of substituents on:**

- **B ring:**
  - F reduces 0.6-0.7 $pK_a$ units (N1, N2)
  - Cl reduces 0.7-0.8 $pK_a$ units (N2 only)

- **A ring:**
  - F or Cl (R1) reduces 0.8 $pK_a$ units (N1)
  - F or Cl reduces 0.2 $pK_a$ units (N2 only)

Biological results: Resistance profile

Activity of compounds 1 and 2 against *T. brucei* 427WT and the isometamidium-resistant strain ISMR1 (dyskinetoplastic strain)

<table>
<thead>
<tr>
<th>Compounds</th>
<th><em>T. b. brucei</em> 427WT</th>
<th><em>T. b. brucei</em> ISMR1</th>
<th>Resistance Factor vs. Tb247WT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC$_{50}$ (µM) ± SEM</td>
<td>EC$_{50}$ (µM) ± SEM</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.83 ± 0.08 a</td>
<td>105.3 ± 3.2</td>
<td>127 ***</td>
</tr>
<tr>
<td>2</td>
<td>0.220 ± 0.002 a</td>
<td>29.0 ± 0.7</td>
<td>132 ***</td>
</tr>
<tr>
<td>Isometamidium</td>
<td>0.016 ± 0.001</td>
<td>1464 ± 94</td>
<td>92522 ***</td>
</tr>
</tbody>
</table>

The compounds were significantly less effective against the dyskinetoplastic strain, with 127- and 132-fold increases in EC$_{50}$ values, respectively, indicating that the absence of kDNA has made the cells resistant to the test compounds.
Biological results: Alteration of the cell cycle of *T. brucei*

In the *T. brucei* cell cycle, replication and division of kDNA necessarily precedes nuclear division, and compounds that directly impact on kDNA are thus expected to interfere with cell division.

Indeed, both compounds dose-dependently reduced *T. brucei* growth rates and, at concentrations above EC$_{50}$, appeared to induce growth arrest after 24 h.

Biological results: Alteration of the cell cycle of *T. brucei*

Histograms of flow cytometric analysis

DNA content is clearly modified when treated with the compounds. Using flow cytometry we observed that our compounds act in the S-phase, when DNA synthesis is produced. So, compounds 1 and 2 impact on kDNA by interfering with cell division.

Binding analysis: Compound 2 inhibits the binding of high mobility group proteins HMGA1a and HMGB1 to AT-rich DNA

Using SPR technique for a binding assay, we observed how at crescent concentration of protein the detection of the DNA-protein complex exhibit a linear relation. Whereas in the competition assay we observe that introduction of the inhibitor reduce the formation of the DNA-protein complex. We showed that compound 2 can displace HMG-box containing proteins essential for kDNA function from their kDNA binding sites.

Biological results: Fluorescent compound 2 accumulates in the mitochondrion of trypanosomes

Fluorescence technique shows compound 2 is accumulated inside the parasite in the mitochondrion of trypanosomes and after different times of interaction several damages in the cell are observed.

Biological results: Compounds 1 and 2 cause destruction of the kinetoplast DNA network

<table>
<thead>
<tr>
<th>Drug Free</th>
<th>3 h of 1</th>
<th>3 h of 2</th>
<th>24 h of 1</th>
<th>24 h of 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
</tr>
</tbody>
</table>

TEM images shows the compounds cause ultrastructural abnormalities in the kinetoplast, which were clearly far more severe at 24 h than at 3 h of incubation, when many cells appeared still undamaged.

Biological results: Compounds 1 and 2 show curative activity in a mouse model of first stage HAT

*T. b. rhodesiense* infection (mouse model of stage 1 HAT):
- Compound 1 is 100% curative by ip (4×5 mg/kg/day) and oral dosage (4×50 mg/kg/day)
- Chloro analogue 2 is 100% curative at 4×20 mg/kg/day (ip)
- At lower dosage (4×4 mg/kg/day, ip), 2 increased the mean day of relapse of parasitemia at although no cures were obtained.

Structural analysis: X-ray structure of the DNA–compound 1 complex at 1.25 Å

Drug conformation and interactions

DNA oligonucleotide [AAATTT]$_2$

The crystal structure of compound 1 bounded to an AT-rich DNA was solved at atomic resolution of 1.25 Å.

The drug molecules fill the central part of the minor groove of the duplexes.

The crystal is stabilized in part by the interaction of the central molecule, drug F (pink) with the DNA phosphates of neighboring molecules.

Conclusions

• The introduction of halogens atoms in the structure of compound 1 led to a reduction of basicity of both imidazoline rings.

• Compound 2 with a chlorine atom in position R₄ was the most active against T. brucei.

\[
\begin{align*}
\text{Cl} & \quad \text{HN} \\
\text{H} & \quad \text{N} \\
\text{HN} & \quad \text{NH} \\
\end{align*}
\]

• N-phenylbenzamide bis(2-aminoimidazolinium) compounds 1 and 2 share the same mechanism of action against Trypanosoma brucei, acting specifically on the integrity of the kinetoplast by altering the structure and replication of kDNA.
Acknowledgments