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Link to publisher's version: http://dx.doi.org/10.1016/j.bmcl.2014.10.025


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Synthesis and antiprotozoal activity of oligomethylene- and $p$-phenylene-
$\text{bis}$(methylene)-linked $\text{bis}(\text{+})$-huprines

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ABSTRACT

Molecular dimerization of antiprotozoal compounds may be used to overcome drug resistance. Huprines are a class of 4-aminoquinoline derivatives endowed with trypanocidal and antimalarial activities. We have synthesized a series of dimers of (+)-(7R,11R)-huprine Y with linkers of different length and chemical nature and evaluated their activity against cultured bloodstream forms of Trypanosoma brucei and Plasmodium falciparum, and their cytotoxicity against rat myoblast L6 cells. We also assessed their brain permeability, a requirement in drugs aimed at treating the late stages of infections with these parasites. Most of the new dimers exhibit more potent trypanocidal activity than the parent huprine Y, with IC90 values in the submicromolar range and better selectivity indices (up to 38). They have a predicted ability to cross the blood-brain barrier, but seem to be devoid of significant antimalarial activity. Bis(+)huprines therefore emerge as interesting brain permeable trypanocidal leads.
In the last few years, implementation of prevention and control measures has significantly reduced the burden of tropical protozoan diseases such as human African trypanosomiasis (HAT or sleeping sickness) and malaria. However, approximately 70 million people remain at risk of HAT, and in the case of malaria, half of the world’s population live in countries where the disease is endemic. Thus, these parasitic infections continue to pose a serious health threat, especially in developing regions.1,2

The causative agents of HAT and malaria are the single-celled parasites Trypanosoma brucei gambiense or T. brucei rhodesiense, and several species of the genus Plasmodium, amongst which, P. falciparum is the most common and deadly. The parasites are transmitted through the bite of infected insects, namely Glossina flies (tsetse flies) for HAT and Anopheles mosquitoes for malaria.

In HAT, following an initial hemolymphatic phase, parasites can cross the blood–brain barrier (BBB) and infect the central nervous system (CNS), leading to severe neurological symptoms. Without treatment, death is inevitable when the disease has reached this late stage. In malaria, the parasites multiply initially in the liver, and then in the bloodstream. In severe cases, they can become sequestered within brain capillaries, particularly in children, causing the so-called cerebral malaria, frequently with fatal consequences.

Current options to reduce the burden of HAT and malaria are far from ideal.3–5 There is no licensed vaccine for either infection, with vector control and public health measures being the main means of prevention. Currently registered drugs are problematic, with toxicity and resistance being major problems. For example, although five drugs have been approved for the treatment of HAT (pentamidine, suramin, melarsoprol, nifurtimox and eflornithine), their activity can be stage and/or species specific, they display a range of toxic side effects, and require strict and complicated parenteral administration regimens.5 This type of specialized infrastructure is often unavailable in the poor rural settings where HAT is endemic. Drug resistance continues to emerge and undermine clinical effectiveness. Increased resistance has been observed for the trypanocidal agent melarsoprol. In the case of malaria, chloroquine is no longer widely effective and rising resistance against the current front line drug artemisinin is a significant threat to global health. Overall, there is an acute need to develop novel drugs for HAT and malaria that can circumvent the limitations of existing therapies.

Several approaches have been proposed to speed up the antiprotozoal drug pipeline. These include high-throughput screening of large compound libraries, new strategies to functionally validate novel druggable targets involved in key steps of the parasite life-cycle,7,10 or the simultaneous inhibition of two or more key biological targets with combination therapies or multitarget-directed ligands.11,13 Increasingly, the search for novel antiprotozoal agents also involves the repositioning of existing drugs registered for other applications14 or the synthesis of new chemical entities endowed with antiprotozoal activity.15–17

In recent years, new compounds bearing the 4-amino-7-chloroquinoline core of chloroquine, or other aminoquinoline moieties, have been assessed as novel trypanocidal or antimalarial agents, or as dual agents endowed with both activities.18–21 The development of single molecules that have potency against different protozoan diseases (such as HAT and malaria) has been regarded as an feasible approach, with potential economic savings.22 We recently reported that the aminoquinoline derivatives huprines, a structural class initially developed as inhibitors of the enzyme acetylcholinesterase, are moderately effective and selective trypanocidal agents, with some also being active against a chloroquine-resistant strain of P. falciparum.23,24 In particular, the 4-amino-7-chloroquinoline derivative 1 (huprine Y, Scheme 1) exhibited the lowest IC₅₀ value of the series against T. brucei (IC₅₀ = 0.61 µM; IC₉₀ = 2.94 µM), with one of the best selectivity indices over rat myoblast L6 cells (SI = 13) among the entire set of tested huprines.25

Molecular dimerization of compounds with known antiprotozoal activity constitutes a strategy that can be used to overcome drug resistance.26 This approach has proven successful for dimers of 4-aminoquinolines, in which the two constituting units were connected through linkers of different length or containing different functional groups.26–29

Here, we report the synthesis of dimers of huprine Y, in which the two huprine moieties have been connected through oligomethylene linkers of different length, or with a p-phenylene-bis(methylene) tether. To this end, enantiopure (+)-(7R,11R)-huprine Y [(7R,11R)-1, Scheme 1], the least active enantiomer in terms of acetylcholinesterase inhibition activity,30–32 has been used. The dimeric bist(+)-huprines have been tested against cultured bloodstream forms of T. brucei and P. falciparum, and their cytotoxicity against mammalian cells and brain permeability has been assessed.

The synthesis of hexa-, octa-, deca-, and dodeca-methylene linked bist-huprines (+)-2a–d and the p-phenylene-bis(methylene)-linked bist-huprine (+)-2e was carried out by reaction of 2 equivalents of (+)-(7R,11R)-huprine Y with 1 equivalent of the corresponding o,o-di-haloalkane, using KOH as the base in DMSO at room temperature for three days (Scheme 1). After silica gel column chromatography purification, bist(+)-huprines (+)-2a–d were obtained in moderate yields (21–50% yields, whereas (+)-2e was obtained in a lower yield (11%) along with the byproduct resulting from the monoalkylation of (+)-1 (12% yield).

Bist-huprines (+)-2a–e were converted into the corresponding dihydrochlorides for their chemical characterization (specific rotation, melting point, IR, 1H and 13C NMR, HRMS, and elemental analysis) and biological profiling.33

![Scheme 1. Synthesis of bist-huprines (+)-2a–e.](image-url)

**Scheme 1.** Synthesis of bist-huprines (+)-2a–e.

Bist-huprines (+)-2a–e were first tested in vitro against cultured bloodstream forms of T. brucei. All of the bist-huprines exhibited IC₅₀ values in the range 0.50–0.89 µM. Their IC₉₀ values of around 1 µM (0.73–1.09 µM) (Table 1), were significantly lower than the parent huprine Y (IC₅₀ = 2.94 µM).
Given the narrow range of potencies of the different bis-huprines, the length of the linker or the presence of a benzene ring within the linker do not seem to have a strong influence on the trypanocidal activity of bis(+-)huprines. Thus, the increased trypanocidal potency of bis-huprines, relative to huprine Y, might be ascribed to the dimerization strategy, even though the mechanisms responsible for inhibition of trypanosome growth or for the enhanced activity are not known.

*Bis*-huprines (+)-2a–e were also evaluated against the chloroquine-resistant K1 strain of *P. falciparum*. Even though some huprines have been reported to exhibit moderately potent antiplasmodial properties,21 huprine Y did not exhibit significant activity (IC50 > 10 µM). Huprine Y bears the 4-amino-7-chloroquinoline moiety, thought to be an antimalarial pharmacophore responsible for inhibition of haem dimerization.20,34 Since dimerization of other 4-aminoquinoline compounds increased antiplasmodial potency and/or overcame the chloroquine resistance mechanism,26–29 we hypothesized that dimerization of huprine Y to bis-huprines (+)-2a–e might also enhance activity. However, no noticeably increased antiplasmodial potency was observed for the dimeric compounds, which exhibited IC50 values > 5 µg/mL (i.e. > 6–7 µM), much higher than that of artemisinin (IC50 = 91 nM) used in this assay as a positive control. The improvement of potency against chloroquine resistant strains of *P. falciparum* of other bis(4-aminoquinoline) derivatives relative to the corresponding monomeric compounds has been ascribed mainly to the doubling of the number of protonatable nitrogen atoms in the dimers, which might lead to more efficient trapping in the acidic digestive vacuole of the parasite and prevention of heme polymerization.25,29 The failure of *bis*-huprines to show antiplasmodial activity might be indicative of the fact that these compounds cannot hit the biological target of chloroquine and other 4-aminoquinoline derivatives despite their structural similarity. Indeed, we have recently found that the parent huprine Y, unlike chloroquine, shows no inhibition of β-haematin formation, whereas several huprine analogues that possess antiplasmodial activity are effective inhibitors of β-haematin formation (unpublished results).

### Table 1

<table>
<thead>
<tr>
<th>Compd</th>
<th><em>T. brucei</em></th>
<th><em>T. brucei</em></th>
<th>L6 cells</th>
<th>SF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC50 µM</td>
<td>IC50 µM</td>
<td>IC50 µM</td>
<td></td>
</tr>
<tr>
<td>(+)-2a</td>
<td>0.89 ± 0.02</td>
<td>1.09 ± 0.01</td>
<td>1.61 ± 0.07</td>
<td>1.8</td>
</tr>
<tr>
<td>(+)-2b</td>
<td>0.52 ± 0.01</td>
<td>0.74 ± 0.02</td>
<td>4.92 ± 0.15</td>
<td>9.5</td>
</tr>
<tr>
<td>(+)-2c</td>
<td>0.50 ± 0.01</td>
<td>0.73 ± 0.01</td>
<td>7.71 ± 0.70</td>
<td>15.4</td>
</tr>
<tr>
<td>(+)-2d</td>
<td>0.76 ± 0.03</td>
<td>0.99 ± 0.05</td>
<td>28.5 ± 2.9</td>
<td>37.5</td>
</tr>
<tr>
<td>(+)-2e</td>
<td>0.57 ± 0.02</td>
<td>0.75 ± 0.02</td>
<td>16.1 ± 0.3</td>
<td>28.2</td>
</tr>
<tr>
<td>huprine Y</td>
<td>0.61 ± 0.03</td>
<td>2.94 ± 0.20</td>
<td>7.80 ± 0.47</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td>SF: Selectivity index is the ratio of cytotoxic to trypanocidal IC50 values.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*In vitro* activity against bloodstream form of *T. brucei* (pH 7.4) and rat myoblast L6 cells expressed at the concentration that inhibited growth by 50% (IC50) and 90% (IC90, for trypanocidal activity). Data are the mean of triplicate experiments ± SEM.

### Table 2

<table>
<thead>
<tr>
<th>Compd</th>
<th><em>P</em> (10⁻³ cm s⁻¹)</th>
<th>Prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-2a</td>
<td>11.1 ± 0.3</td>
<td>CNS+</td>
</tr>
<tr>
<td>(+)-2b</td>
<td>13.9 ± 1.0</td>
<td>CNS+</td>
</tr>
<tr>
<td>(+)-2c</td>
<td>8.7 ± 1.5</td>
<td>CNS+</td>
</tr>
<tr>
<td>(+)-2d</td>
<td>17.4 ± 0.7</td>
<td>CNS+</td>
</tr>
<tr>
<td>(+)-2e</td>
<td>8.3 ± 0.6</td>
<td>CNS+</td>
</tr>
<tr>
<td>huprine Y</td>
<td>23.8 ± 2.7</td>
<td>CNS+</td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± SD of three independent experiments.

*Take from ref. 32.*

### Notes

In summary, we report the synthesis of a series of dimeric bis(4-aminoquinoline) derivatives, which are composed of two units of (+)-(7R,11R)-huprine Y connected through oligomethylene linkers of different length or a p-phenylene-bis(methylene) linker. We also describe the assessment of the different bis(+-)huprines on the growth of bloodstream forms of *T. brucei* and *P. falciparum*, and of rat skeletal myoblast L6 cells, as well as their BBB permeability. All of the bis(+-)huprines exhibited potent trypanocidal activity, with IC50 and IC90 values in the submicromolar range. However, they did not exhibit significant antiplasmodial activity. As trypanocidal agents, bis(+-)huprines are more potent than monomeric huprine Y and...
some of them, particularly the dodecamethylene- and p-phenylene-bis(methylene)-linked dimers (+)-2d and (+)-2e, are less cytotoxic and, hence, more selective for T. brucei over rat L6 cells growth inhibition than huprine Y. All the bis(+)-huprines have been predicted to have the ability to cross the BBB, thereby being potentially useful for the treatment of late-stage HAT. Overall, bis(+)-huprines (+)-2d and (+)-2e emerge as interesting lead compounds for further trypanocidal drug discovery endeavours.

Acknowledgments

This work was supported by Ministerio de Ciencia e Innovación (MICINN) (CTQ2011-22433) and Generalitat de Catalunya (GC) (2014SGR52). JMK acknowledges funding support from the Wellcome Trust (Grant number WT084175). A fellowship from GC to I.S. is gratefully acknowledged. SYG is grateful to the Commonwealth Scholarship Secretariat, UK and to the Government of Ghana for financial support.

Supplementary data

Supplementary data (synthetic procedures and chemical characterization of bis(+)-huprines and biological methods) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j...
for \((\text{C}_6\text{H}_9\text{Cl}_3\text{N}_4 \cdot 2\text{HCl} \cdot 1.25\text{H}_2\text{O})\): C 66.46%, H 7.34%, N 6.74%;
found: C 66.63%, H 7.66%, N 6.23%.