Drug targeting of natural products: the example of antileishmanial quinolines

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Drug targeting of natural products: the example of antileishmanial quinolines
Abstract:

Quinolines of natural origin have shown antileishmanial activities on several experimental leishmaniasis models. However, a classical daily treatment with 2-n-propylquinoline (2-n-PQ) on five consecutive days in mice model is not sufficient to cure the mice infected with *Leishmania donovani* as the activity requires a 10-day treatment duration whatever the route (oral, parenteral) because of a short half-life elimination of the drug. Therefore, 2-n-PQ derivatives were bound to soluble polysaccharides to improve their solubility, delay their elimination half-life and therefore enhance the activity. *In vitro*, the most active conjugate was the dextran-2PQA conjugate. However, this system did not allow a sufficient release of the active principle explaining the lack of *in vivo* activity.

Another approach consisted in administering 2-n-PQ intravenously. Two systems were successful both *in vitro* and *in vivo*: a liposomal formulation named 2-n-PQ-LIP and a hydroxypropyl beta-cyclodextrin inclusion complex designated as 2-n-PQ-HPC. The most interesting one was the liposomal formulation, active on the *L. donovani* Balb/c mouse model, by reducing the parasite burden by more than 80% after an intravenous treatment regimen of 3 mg equivalent 2-n-PQ/kg/day given on five consecutive days. These formulations should be studied further on other leishmaniasis models and for toxicological considerations.
Infectious diseases caused by Euglenozoa parasites from the genus *Leishmania sp.*

**Human leishmaniases**

**CUTANEOUS / MUCOCUTANEOUS FORMS**

- **Localized cutaneous**
- **Diffuse cutaneous**
- **Muco-cutaneous**

**VISCERAL FORM**

- 350.10^6 persons at risk (Africa, South America, Asia, Southern Europe)
- 12.10^6 cases worldwide and 2.10^6 new cases per year (500 000 new cases of VL in India, Bangladesh, Nepal, East Africa)
Life cycle of *Leishmania sp.*

- **Promastigote form in the sandfly**

- **Amastigote form in macrophage**

**Sandfly**
- *Leishmania donovani* → Anthroponotic disease
- *Leishmania infantum* → Zoonotic disease
Limitations of current treatments

- Meglumine antimoniate (Glucantime®)
- Sodium stibogluconate (Pentostam®)
- Miltefosine (Impavido®)
- Amphotericin B (liposomal → AmBisome®)
- Paromomycin

Two limitations:
- Drug toxicity
  - Need of specific drugs
- Drug resistance
  - Need of new chemical series
- Ethnopharmacological study in Bolivia

- Dialog between traditionnal practionners and scientists

→ Identification of bark of *Galipea longiflora* (Rutaceae)

→ Traditionnaly used against Cutaneous Leishmaniasis (CL) lesions

→ Purification of 2-substituted quinolines

→ Active by oral route on leishmaniasis experimental animal models
**In vivo** active 2-substituted-quinolines isolated from *G. longiflora*

- 2-**n**-propylquinoline
  - Active against CL and VL

- Chiminan B
  - Active against CL

- Chimanine D
  - Active against CL

- 2-phenylquinoline
  - Active against CL

- Cusparine
  - Active against CL

- 2-(3,4-dimethoxyphenylethyl) quinoline
  - Active against CL

- 2-(3,4-methylenedioxyethyl) quinoline
  - Active against CL

- Skimmianine
  - Active against CL
In vivo active 2-substituted-quinolines isolated from *G. longiflora*

- **2-n-propylquinoline**
  - Active against CL and VL

- **Chimanin B**
  - Active against CL

- **Chimanine D**
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- **2-phenylquinoline**
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- **Cusparine**
  - Active against CL

- **2-(3,4-dimethoxyphenylethyl)quinoline**
  - Active against CL

- **2-(3,4-methylendioxyethyl)quinoline**
  - Active against CL

- **skimmianine**
  - Active against CL
Major data about 2-\(n\)-PQ

**Chemical synthesis**

\(\rightarrow\) Easy: two steps and good yield

**Antileishmanial activity /toxicity**

\(\rightarrow\) 2-\(n\)-PQ is active by intraperitoneal and oral routes on experimental visceral leishmaniasis models (\textit{L. amazonensis}, \textit{L. donovani}) at 10-12 mg/kg/day \(\times\) 10 (Nakayama et al., AAC, 2005)

\(\rightarrow\) Absence of toxicity after oral/ip administration at 1g/kg in mice

**Mechanism of action of 2-substituted quinolines on \textit{Leishmania}**

\(\rightarrow\) Alteration of parasite bioenergetics (Bompart et al., 2013)

\(\rightarrow\) Disruption of mitochondrial electrochemical potential

\(\rightarrow\) Alkalization of acidocalcisomes

\(\rightarrow\) Partial inhibition of ergosterol biosynthetic pathway (Bompart et al., 2013)
Comparative data of pharmacokinetics between antileishmanial quinolines

<table>
<thead>
<tr>
<th>Substituted-quinolines</th>
<th>Compounds</th>
<th>PK after oral administration $T_{1/2}$ absorption</th>
<th>PK after oral administration $T_{1/2}$ elimination</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-amino-</td>
<td>Primaquine</td>
<td>1 h (human)</td>
<td>6.3 h (human)</td>
</tr>
<tr>
<td>8-amino-</td>
<td>Sitamaquine</td>
<td>1.5-3 h (human)</td>
<td>26.1 h (human)</td>
</tr>
<tr>
<td>8-amino-</td>
<td>Tafenoquine</td>
<td>1 h (human)</td>
<td>16.4 days (human)</td>
</tr>
<tr>
<td>2-substituted-</td>
<td>2-$n$-PQ</td>
<td>15 min (rat)</td>
<td>1.6 h (rat)</td>
</tr>
</tbody>
</table>

**Drawback**

→ Short half-life of elimination
Drug targeting as a strategy to enhance the 2-n-PQ biodistribution via intravenous route

Intravenous route

→ Water-soluble conjugates
→ Liposomes
→ Cyclodextrins
Design of water-soluble polymers for iv route

→ Developing an intravenous formulation as a prolonged drug release system for intravenous administration

2-n-PQ cannot be substituted to get polymers

→ Necessity to synthesize an active derivative

2-Propylquinoline (2-PQ)  

\[
\text{2-Propylquinoline (2-PQ)}
\]

2-(2-amino-2-enyl)quinoline (2-PQA) = active derivative of 2-PQ

\[
\text{2-(2-amino-2-enyl)quinoline (2-PQA)}
\]

Conjugation of 2-PQA with water soluble bio-polymer such as polyglucose, gum arabic and dextran
What is the rationale for drug delivery?

- The polymer protects the drug from enzymatic and chemical degradation

- The polymer reduces the rate of elimination of the drug owing to its high molecular weight, increasing the residence time of the drug

- The conjugation of the drug to the polymer promotes targeted drug delivery mainly to the sites in the body with increased capillary permeability such as inflamed tissues
Design of water-soluble polymers

2-propyquinoline  
(E)-3-(quinolin-2-yl)prop-2-en-1-amine  
mono((E)-3-(quinolin-2-yl)prop-2-en-1-aminium) dichloride

Synthesis of compound 2PQA.2HCl

1) K$_2$CO$_3$, (PPh$_3$)$_2$PdCl$_2$  
Dioxanne/H$_2$O, Reflux

2) AcCl/MeOH, AcOEt  
49% (2 steps)

mono((E)-3-(quinolin-2-yl)prop-2-en-1-aminium) dichloride
Conjugation of 2PQA to oxidized polyglucose (also oxidized dextran)

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Periodate equivalent (%)</th>
<th>Theoretical Degree of oxidation (%)</th>
<th>Observed Degree of oxidation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG</td>
<td>30</td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td>PG</td>
<td>50</td>
<td>50</td>
<td>49</td>
</tr>
<tr>
<td>Dextran</td>
<td>20</td>
<td>20</td>
<td>18</td>
</tr>
</tbody>
</table>

**Periodate oxidation of polyglucose (PG) and dextran**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Theoretical loading (wt%)</th>
<th>Actual loading (wt%)</th>
<th>Incorporation efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG (50% oxidized)</td>
<td>20</td>
<td>18</td>
<td>90</td>
</tr>
<tr>
<td>PG (30% oxidized)</td>
<td>20</td>
<td>16</td>
<td>80</td>
</tr>
<tr>
<td>Dextran (20% oxidized)</td>
<td>20</td>
<td>18</td>
<td>90</td>
</tr>
</tbody>
</table>

**Incorporation efficiency of 2PQA in polyglucose (PG) and dextran conjugates**
Stability of the PG-2PQA conjugate

UV-visible spectrum of PG-2PQA soon after preparation (a) and after 6 month storage as lyophilized powder stored at 4°C (b)

→ Complete stability after a 6 month storage of the lyophilized powder at 4°C in light-protected glass containers

In vitro PG-2PQA release

Cumulative release of 2PQA from the PG-2PQA conjugate having 16% drug payload (■), and oxidized dextran-2PQA conjugate having 18% drug payload (●) at pH 7.4 at 37°C

→ Hydrolytic susceptibility of the Schiff’s linkage
**In vitro and in vivo antileishmanial activity**

<table>
<thead>
<tr>
<th>Compound /Formulation</th>
<th>In vitro activity on <em>L. donovani</em> IC₅₀ (µg/mL ± SD)</th>
<th>Treatment regimen-iv route for 5 days (mg/kg)</th>
<th>In vivo activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Axenic amastigotes</td>
<td>Intramacrophage amastigotes</td>
<td>No of mice</td>
</tr>
<tr>
<td>OPG-5020-2PQA</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>10</td>
</tr>
<tr>
<td>OPG-3020-2PQA</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>10</td>
</tr>
<tr>
<td>OD-2020-2PQA</td>
<td>&gt; 100</td>
<td>12.52 ± 0.4</td>
<td>10</td>
</tr>
<tr>
<td>30% OPG</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>10</td>
</tr>
<tr>
<td>20% OD</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>10</td>
</tr>
<tr>
<td>2PQA</td>
<td>20.62 ± 1.73</td>
<td>12.53 ± 0.62</td>
<td>10</td>
</tr>
<tr>
<td>2PQA.2HCl</td>
<td>0.78 ± 0.09</td>
<td>1.24 ± 0.24 (5 µM)</td>
<td>10</td>
</tr>
<tr>
<td>AmBisome®</td>
<td>2.54 ± 0.70</td>
<td>1.51 ± 0.22</td>
<td>1</td>
</tr>
<tr>
<td>Control (vehicle)</td>
<td>-</td>
<td>&gt; 100 µg/mL</td>
<td>0.2 mL</td>
</tr>
</tbody>
</table>

a versus control mice, P<0.005, OPG: Oxidized polyglucose, OD: Oxidized dextran, OPG-5020: 50% oxidized polyglucose with 20% drug. OPG-3020: 30% oxidized polyglucose with 20% drug, OD-2020: 20% oxidized dextran with 20% drug.

→ Water-soluble conjugates: a not successful strategy because drug release

→ Infratherapeutic concentrations
Design of a 2-n-PQ liposomal formulation for intravenous route → visceral leishmaniasis

2-n-PQ drawback → Lipophilic nature making it difficult to prepare an intravenous formulation

Aim → Developing a formulation for intravenous administration as a nanosystem concentrating 2-n-PQ to the site where parasites are located, mainly in the liver

→ Encapsulation of 2-n-PQ in liposomes
# Optimization studies of 2-*n*-PQ liposomal formulation

<table>
<thead>
<tr>
<th>S. No</th>
<th>Egg PC (%)</th>
<th>Chol (%)</th>
<th>2-PQ (%)</th>
<th>Size (nm)</th>
<th>EE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>---</td>
<td>---</td>
<td>161 ± 2</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>10</td>
<td>---</td>
<td>172 ± 2</td>
<td>---</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>20</td>
<td>---</td>
<td>175 ± 2</td>
<td>---</td>
</tr>
<tr>
<td>4</td>
<td>70</td>
<td>30</td>
<td>---</td>
<td>182 ± 2</td>
<td>---</td>
</tr>
<tr>
<td>5</td>
<td>95</td>
<td>---</td>
<td>5</td>
<td>174 ± 2</td>
<td>41</td>
</tr>
<tr>
<td>6</td>
<td>90</td>
<td>---</td>
<td>10</td>
<td>160 ± 3</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>80</td>
<td>---</td>
<td>20</td>
<td>148 ± 3</td>
<td>13</td>
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<tr>
<td>8</td>
<td>85</td>
<td>10</td>
<td>5</td>
<td>148 ± 4</td>
<td>33</td>
</tr>
<tr>
<td>9</td>
<td>80</td>
<td>10</td>
<td>10</td>
<td>156 ± 3</td>
<td>53</td>
</tr>
<tr>
<td>10</td>
<td>70</td>
<td>10</td>
<td>5</td>
<td>164 ± 4</td>
<td>28</td>
</tr>
<tr>
<td>11</td>
<td>75</td>
<td>20</td>
<td>5</td>
<td>163 ± 4</td>
<td>47</td>
</tr>
<tr>
<td>12</td>
<td>70</td>
<td>20</td>
<td>10</td>
<td>158 ± 3</td>
<td>34</td>
</tr>
<tr>
<td>13</td>
<td>60</td>
<td>20</td>
<td>20</td>
<td>158 ± 3</td>
<td>30</td>
</tr>
<tr>
<td>14</td>
<td>65</td>
<td>30</td>
<td>5</td>
<td>146 ± 4</td>
<td>61</td>
</tr>
<tr>
<td>15</td>
<td>60</td>
<td>30</td>
<td>10</td>
<td>144 ± 2</td>
<td>5</td>
</tr>
<tr>
<td>16</td>
<td>50</td>
<td>30</td>
<td>20</td>
<td>158 ± 4</td>
<td>5</td>
</tr>
</tbody>
</table>
In vitro and in vivo evaluation of 2-n-PQ liposomal formulation and 2-n-PQ-AmB liposomal formulation on the Leishmania donovani / Balb/c mice model

<table>
<thead>
<tr>
<th>Compound / Formulation</th>
<th>In vitro activity on L. donovani</th>
<th>In vivo activity</th>
<th>Reduction of parasite burden (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC₅₀ (µM ± SD)</td>
<td>Number of mice</td>
<td>Route</td>
</tr>
<tr>
<td>Axenic amastigotes</td>
<td>Intramacrophage amastigotes</td>
<td>Regimen</td>
<td></td>
</tr>
<tr>
<td>Liposomal 2PQ</td>
<td>3.10±0.25</td>
<td>3 mg/kg 2PQ x 5 days</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>5.84±0.31</td>
<td>1.5 mg/kg 2PQ x 5 days</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.75 mg/kg 2PQ x 5 days</td>
<td>8</td>
</tr>
<tr>
<td>Liposomal 2PQ+AmB 6.08±0.85 Eq 2PQ</td>
<td>13.5±1.93 Eq 2PQ</td>
<td>0.75 mg 2PQ + 0.006 mg AmB/kg x 5</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.37 mg 2PQ + 0.003 mg AmB/kg x 5</td>
<td>8</td>
</tr>
<tr>
<td>AmBisome®</td>
<td>2.54±0.70</td>
<td>1 mg AmB/kg x 5 days</td>
<td>8</td>
</tr>
<tr>
<td>Blank liposomes</td>
<td>Inactive</td>
<td>0.25 mg AmB/kg x 5 days</td>
<td>8</td>
</tr>
<tr>
<td>2PQ</td>
<td>&gt; 100</td>
<td>0.006 mg AmB/kg x 5 days</td>
<td>8</td>
</tr>
<tr>
<td>Control (vehicle)</td>
<td>Inactive</td>
<td>Same suspension</td>
<td></td>
</tr>
</tbody>
</table>

³ Significant versus control mice: P<0.05

→ Liposomal 2-n-PQ: active at a total dose of 15 mg/kg

→ No synergy in vitro between AmB and 2-n-PQ but slight synergy in vivo
Design of a 2-\(n\)-PQ formulation for intravenous route \(\rightarrow\) disseminated leishmaniasis

\(2-n\)-PQ drawback \(\rightarrow\) Lipophilic nature making it difficult to prepare an intravenous formulation

Aim \(\rightarrow\) Getting a hydroxypropyl-\(\beta\)-cyclodextrin (HPC) formulation \(\rightarrow\) 2-\(n\)-PQ-HPC formulation

\(\rightarrow\) Soluble enough for intravenous administration

\(\rightarrow\) Stable

\(\rightarrow\) Suitable for the treatment of experimental leishmaniasis
In vitro activity of the 2-n-PQ-HPC formulation on L. donovani

The hydroxypropyl-β-cyclodextrin (HPC) formulation significantly enhanced the in vitro activity of 2-n-PQ

<table>
<thead>
<tr>
<th>Compound/formulation</th>
<th>In vitro activity on L. donovani IC₅₀ (μM ±SD) [a]</th>
<th>Cytotoxicity Raw 264.7 MTC (μM ±SD) [b]</th>
<th>Selectivity Index (SI) SI= MTC/IC₅₀ [c]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Axenic amastigotes</td>
<td>Intramacrophage amastigotes</td>
<td></td>
</tr>
<tr>
<td>2-n-PQ</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>/</td>
</tr>
<tr>
<td>2-n-PQ-HPC</td>
<td>6.22±0.82</td>
<td>20.01±0.52</td>
<td>&gt;5</td>
</tr>
<tr>
<td>HPC</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>/</td>
</tr>
<tr>
<td>Miltefosine</td>
<td>1.22±0.50</td>
<td>0.85±0.21</td>
<td>&gt;50</td>
</tr>
</tbody>
</table>

[a] Inhibitory Concentration 50% at 72 h, mean ± SD of three independent experiments  
[b] Maximum Tolerated Concentration (MTC) at 72 h  
[c] Selectivity Index (SI) calculated as the ratio of MTC/IC₅₀ on intramacrophage amastigotes
In vivo activity of the 2-n-PQ-HPC formulation on *L. donovani*

Treatment by intravenous route at 10 mg/kg/d x 10 on the *L. donovani/Balb/c* mice model

2-n-PQ-HCP : 2-n-propylquinoline hydroxypropyl-β-cyclodextrin formulation

- Activity similar to those of miltefosine
- No hepatic, renal and blood toxicity
- No activity with a treatment on 5 consecutive days

(Balaraman et al., BP, 2016)

Its *in vitro* interactions with antimonials, amphotericin B and miltefosine were found as additive both in axenic amastigotes and intramacrophage amastigotes.

2-n-PQ-HPC was not able to generate drug resistance after *in vitro* drug pressure since the RI < 4 (1.8).

RI = Resistance Index = \( \text{IC}_{50} \text{ after drug pressure} / \text{IC}_{50} \text{ before drug pressure} \)
Conclusion: from the plant to the formulations

From 2-\textit{n}-PQ, a natural compound, easy to synthesize:

$\rightarrow$ 1 liposomal formulation for intravenous route targeting VL
$\rightarrow$ Active at 3 mg eq 2-\textit{n}-PQ /kg /day x 5 days

$\rightarrow$ 1 hydroxypropyl-\textit{β}-cyclodextrin (HPC) formulation for intravenous route targeting disseminated leishmaniasis
$\rightarrow$ Active at 10 mg eq 2-\textit{n}-PQ /kg /day x 10 days

$\rightarrow$ No success with water-soluble polymers…
Perspectives

→ Determination of the 2-n-PQ amounts in the liver after intravenous administration of the liposomal formulation by using radiolabelled 2-n-PQ
  → Quantification of the drug targeting

→ PK profiles of 2-n-PQ after intravenous administration of liposomal 2-n-PQ and 2-n-PQ-HPC

→ Evaluation of the formulation efficacy on other leishmaniasis experimental models (L. amazonensis, …)

→ Nanoparticulate systems containing 2-n-PQ which are able to remain in the circulation, thereby allowing the drug to reach the parasites in disseminated leishmaniasis → intraveinous route
Acknowledgments