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

K. BALARAMAN[1,2], N. MEKARNIA[1], G. BARRATT[1], S. POMEL[1], S. COJEAN[1], V. KESAVAN[2], A. JAYAKRISHNAN[2], B. FIGADERE[1], P.M. LOISEAU[1]

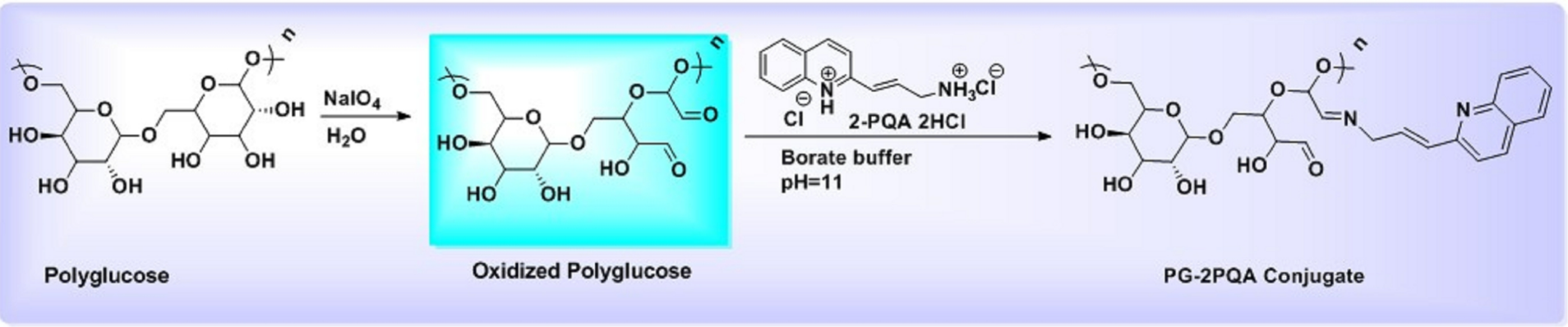
[1]Université Paris-Sud, France

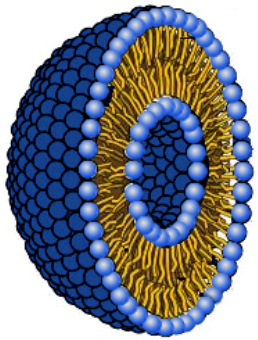
[2]IIT, Madras, India

Laboratory of Excellence in Research on Medication and Innovative Therapeutics

* Corresponding author: philippe.loiseau@u-psud.fr

# 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 treament 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.

# Leishmaniases

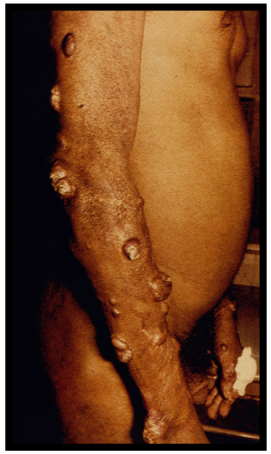
Infectious diseases caused by Euglenozoa parasites from the genus ***Leishmania sp.***

***Human leishmaniases***

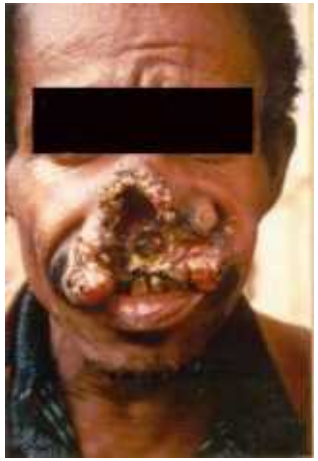
CUTANEOUS / MUCOCUTANEOUS FORMS

VISCERAL FORM

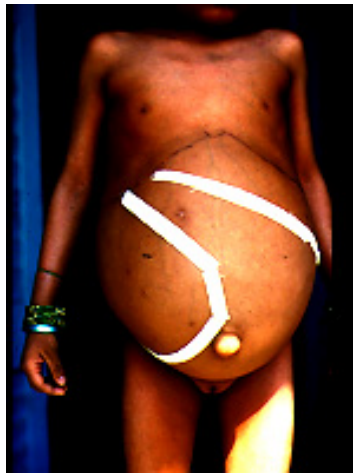
Localized cutaneous



Diffuse cutaneous



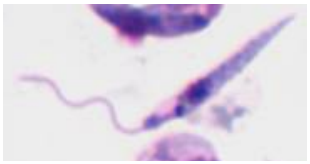
Muco-cutaneous

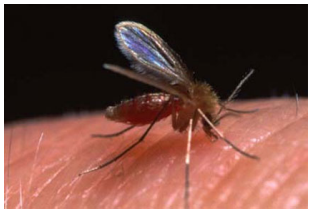


- **350.10$^6$ persons at risk (Africa, South America, Asia, Southern Europe)**
- **12.10$^6$ cases worldwide and 2.10$^6$ new cases per year (500 000 new cases of VL in India, Bengladesh, Nepal, East Africa)**

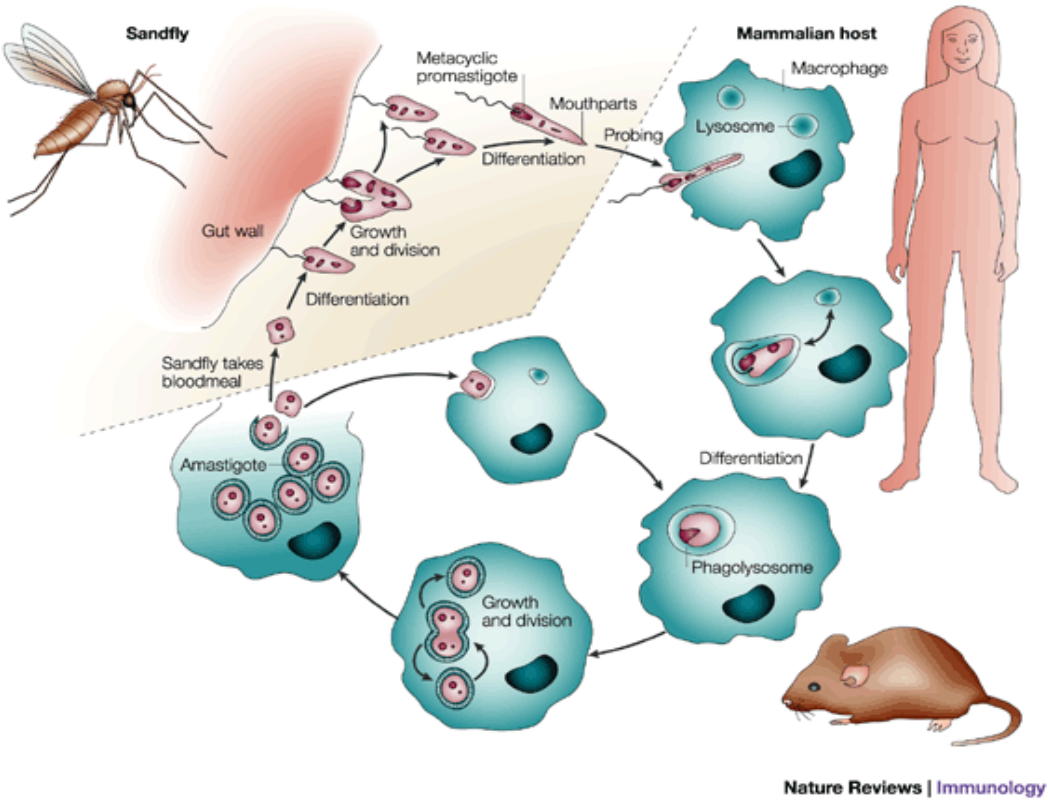
# Life cycle of *Leishmania sp.*

Promastigote form in the sandfly

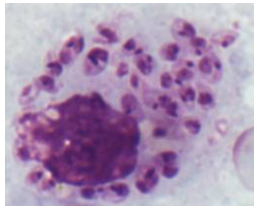




Sandfly



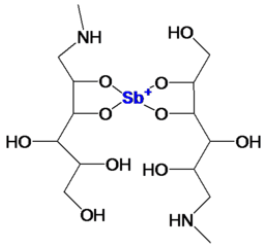

Amastigote form in macrophage



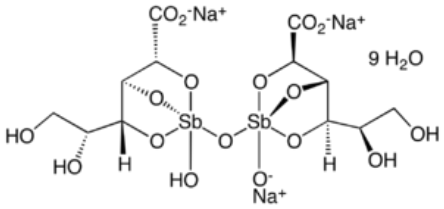
*Leishmania donovani* → Anthroponotic disease

*Leishmania infantum* → Zoonotic disease

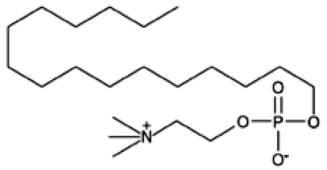
# Limitations of current treatments



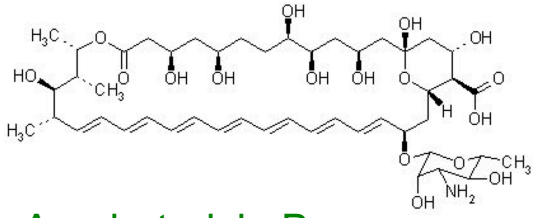
Meglumine antimoniate
(Glucantime®)



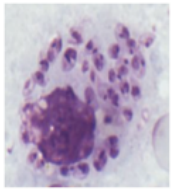
Sodium stibogluconate
(Pentostam®)

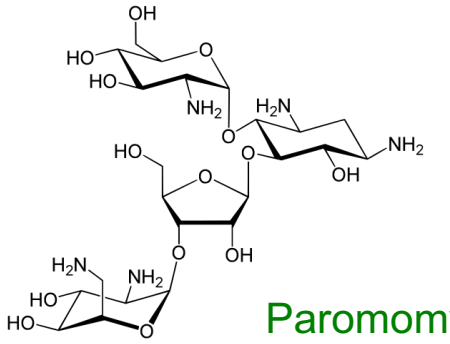


Miltefosine
(Impavido®)



Amphotericin B
(liposomal → AmBisome®)





Paromomycin

Two limitations:

→ Drug toxicity

→ Need of specific drugs

→ Drug resistance

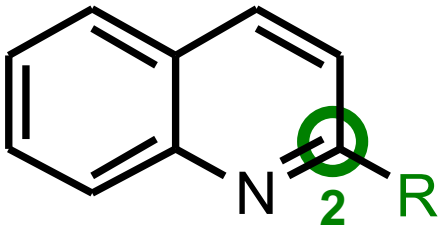
→ Need of new chemical series

# Ethnopharmacology as the source of antileishmanial quinolines

- Ethnopharmacological study in Bolivia

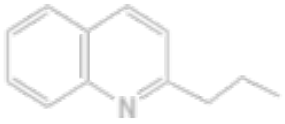
- Dialog between traditionnal practionners and scientists
→ Identification of bark of *Galipea longiflora* (Rutaceae)

→ Traditionnaly used against Cutaneous Leishmaniasis (CL) lesions
→ Purification of 2-substituted quinolines

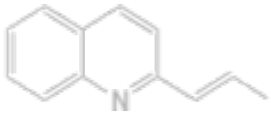



→ Active by oral route on leishmaniasis experimental animal models

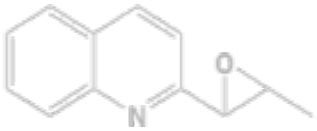
# *In vivo* active 2-substituted-quinolines isolated from *G. longiflora*



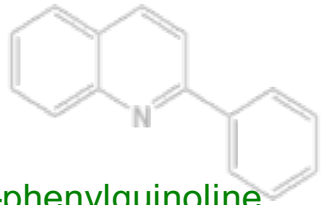
2-*n*-propylquinoline
Active against CL and VL



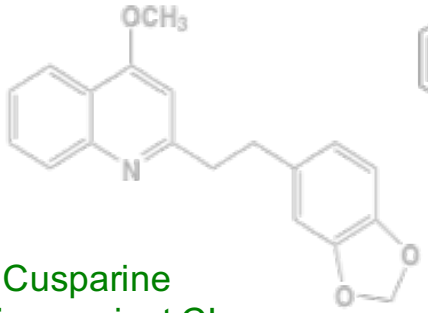
Chimanin B
Active against CL



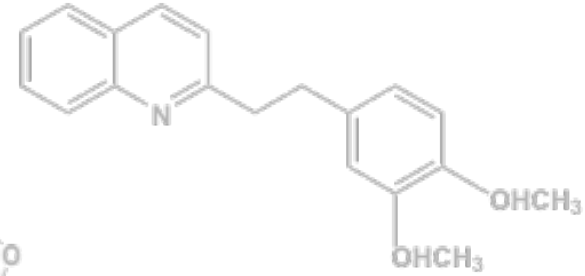
Chimanine D
Active against CL



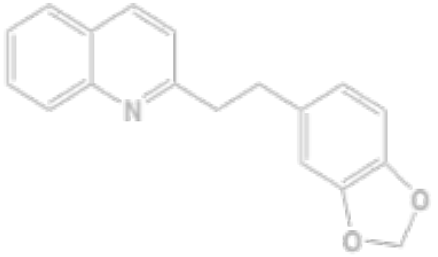
2-phenylquinoline
Active against CL



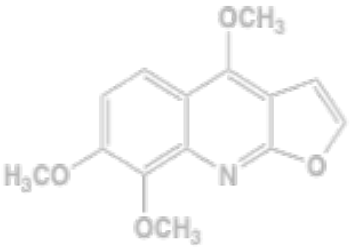

Cusparine
Active against CL




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

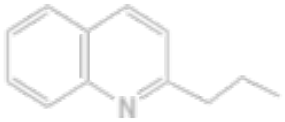



2-(3,4-methylendioxyethyl) quinoline
Active against CL

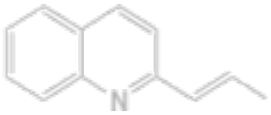



skimmianine
Active against CL

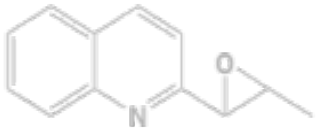
# *In vivo* active 2-substituted-quinolines isolated from *G. longiflora*



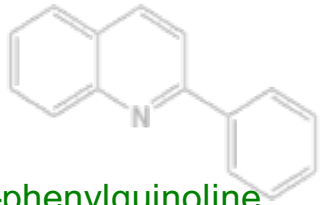
2-*n*-propylquinoline
Active against CL and VL



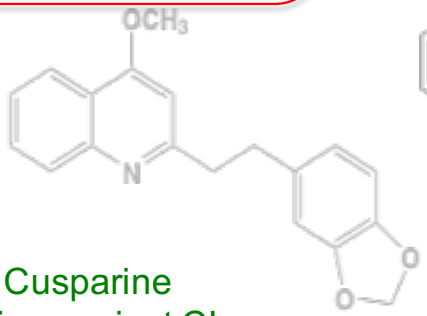
Chimanin B
Active against CL



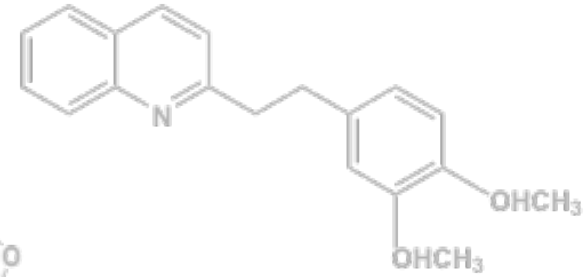
Chimanine D
Active against CL



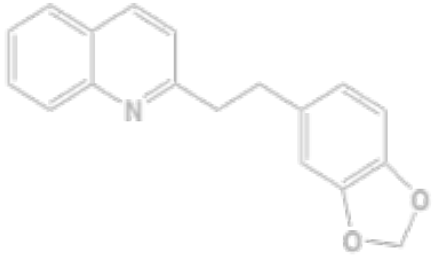
2-phenylquinoline
Active against CL



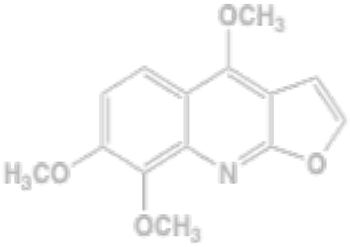
Cusparine
Active against CL



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



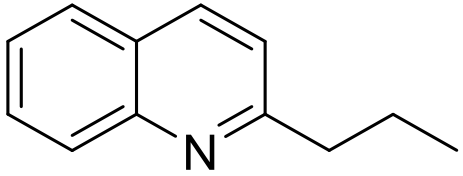
2-(3,4-methylendioxyethyl) quinoline
Active against CL



skimmianine
Active against CL

# Major data about 2-*n*-PQ

## Chemical synthesis




→ Easy: two steps and good yield

## Antileishmanial activity /toxicity

→ 2-*n*-PQ is active by intraperitoneal and oral routes on experimental visceral leishmaniasis models (*L. amazonensis, L. donovani*) at 10-12 mg/kg/day x 10 (Nakayama et al., AAC, 2005)

→ Absence of toxicity after oral/ip administration at 1g/kg in mice

## Mechanism of action of 2-substituted quinolines on *Leishmania*

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

→ **Disruption of mitochondrial electrochemical potential**

→ **Alkalinization of acidocalcisomes**

→ Partial inhibition of ergosterol biosynthetic pathway (Bompart et al., 2013)

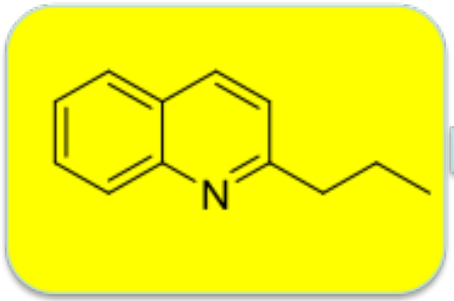
# Comparative data of pharmacokinetics between antileishmanial quinolines

| Substituted-quinolines | Compounds | PK after oral administration $T_{1/2}$ absorption | PK after oral administration $T_{1/2}$ elimination |
|---|---|---|---|
| 8-amino- | Primaquine | 1 h (human) | 6.3 h (human) |
| 8-amino- | Sitamaquine | 1.5-3 h (human) | 26.1 h (human) |
| 8-amino- | Tafenoquine | 1 h (human) | 16.4 days (human) |
| 2-substituted- | 2-*n*-PQ | 15 min (rat) | 1.6 h (rat) |

**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**

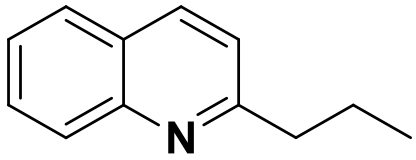
# Design of water-soluble polymers for iv route

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

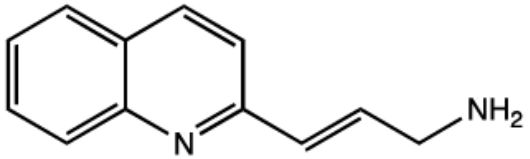
2-*n*-PQ cannot be substituted to get polymers

→ Necessity to synthesize an active derivative

2-Propylquinoline (2-PQ)




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




Conjugation of 2-PQA with water soluble bio-polymer such as polyglucose, gum arabic and dextran

# Design of water-soluble polymers for iv route

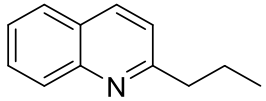
What is the rationale for drug delivery ?

- 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

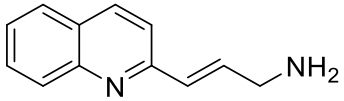
- The conjugation of the drug to the polymer promotes targeted drug delivery mainly to the sites in the body with increased capillary permeability such as inflamed tissues

# Design of water-soluble polymers



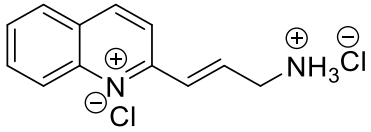
2-propylquinoline

**a**



(*E*)-3-(quinolin-2-yl)prop-2-en-1-amine

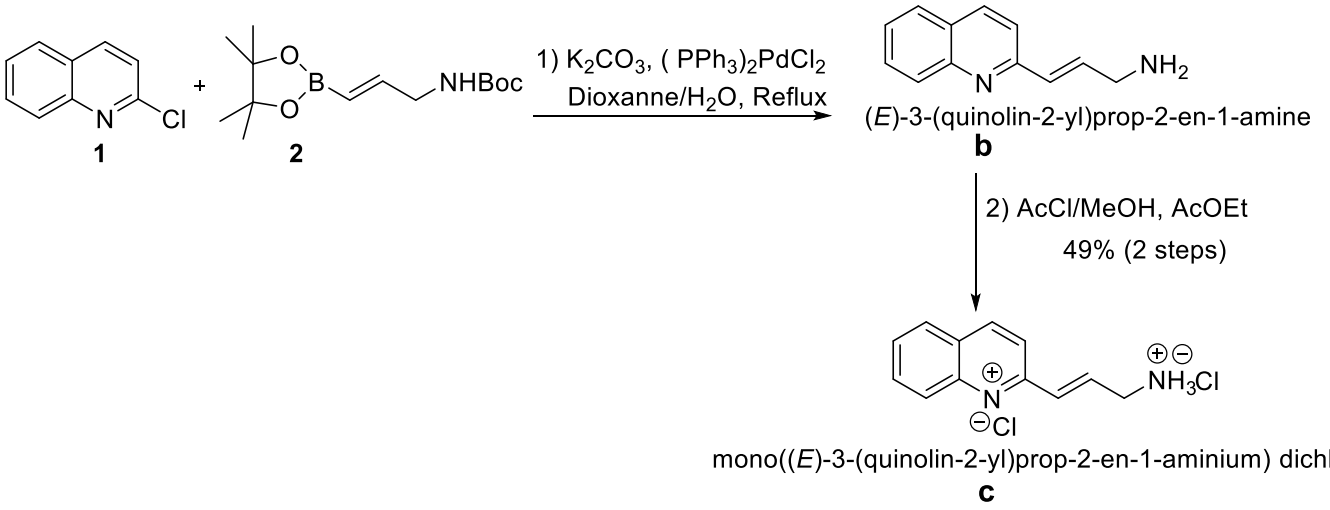
**b**



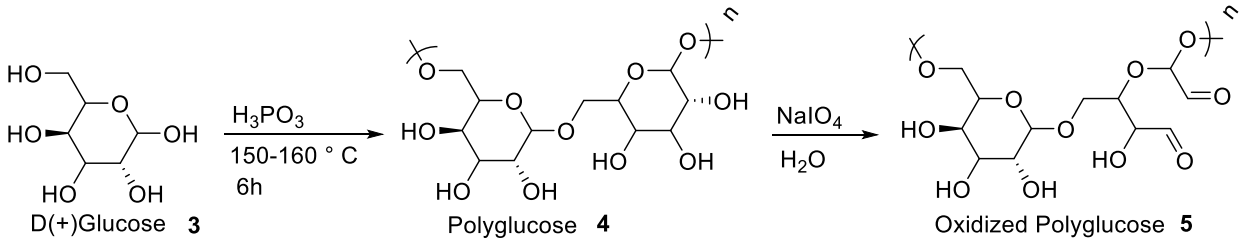
mono((*E*)-3-(quinolin-2-yl)prop-2-en-1-aminium) dichloride

**c**

## Synthesis of compound 2PQA.2HCl



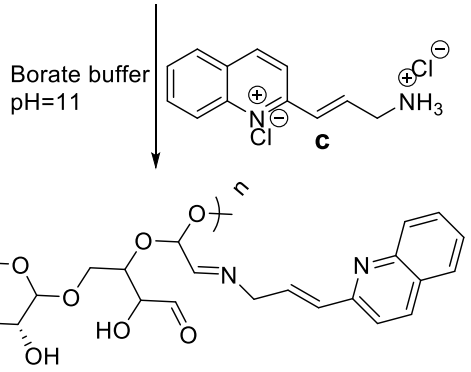
# Conjugation of 2PQA to oxidized polyglucose (also oxidized dextran)




D(+)Glucose **3** Polyglucose **4** Oxidized Polyglucose **5**

| Sample | Periodate equivalent (%) | Theoretical Degree of oxidation (%) | Observed Degree of oxidation (%) |
|---|---|---|---|
| PG | 30 | 30 | 28 |
| PG | 50 | 50 | 49 |
| Dextran | 20 | 20 | 18 |

Periodate oxidation of polyglucose (PG) and dextran




**c**

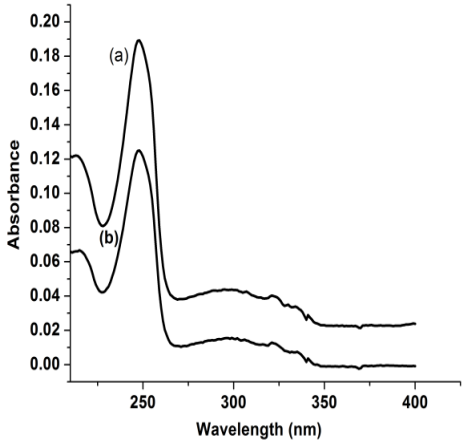
PG-2PQA imine Conjugate **6**

| Sample | Theoretical loading (wt%) | Actual loading (wt%) | Incorporation efficiency (%) |
|---|---|---|---|
| PG (50% oxidized) | 20 | 18 | 90 |
| PG (30% oxidized) | 20 | 16 | 80 |
| Dextran (20% oxidized) | 20 | 18 | 90 |

Incorporation efficiency of 2PQA in polyglucose (PG) and dextran conjugates

## Stability of the PG-2PQA conjugate

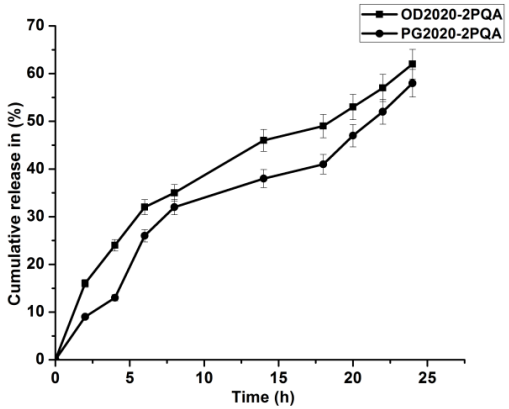
UV-visible spectrum of PG-2PQA soon after preparation (a) and after 6 month storage as lyophilized powder stored at 4°C (b)




→ 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

[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

| Compound /Formulation | *In vitro* activity on *L. donovani* $IC_{50}$ (µg/mL ± SD) | | Treatment regimen-iv route for 5 days (mg/kg) | *In vivo* activity | |
|---|---|---|---|---|---|
| | Axenic amastigotes | Intramacrophage amastigotes | | No of mices | Reduction in parasite burden (%) |
| OPG-5020-2PQA | > 100 | > 100 | 10 | 8 | 4.1 |
| OPG-3020-2PQA | > 100 | > 100 | 10 | 8 | 0 |
| OD-2020-2PQA | > 100 | 12.52 ± 0.4 | 10 | 8 | 4.7 |
| 30% OPG | > 100 | > 100 | 10 | 8 | 2.2 |
| 20% OD | > 100 | > 100 | 10 | 8 | 0.6 |
| 2PQA | 20.62 ± 1.73 | 12.53 ± 0.62 (50 µM) | 10 | 8 | 60.5 [a] |
| 2PQA.2HCl | 0.78 ± 0.09 | 1.24 ± 0.24 (5 µM) | 10 | 8 | 48.8 [a] |
| AmBisome® | 2.54 ± 0.70 | 1.51 ± 0.22 | 1 | 12 | 88.9[a] |
| | | | 0.25 | 12 | 27.1 |
| Control (vehicle) | - | > 100 µg/mL | 0.2 mL | 8 | 0 |

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

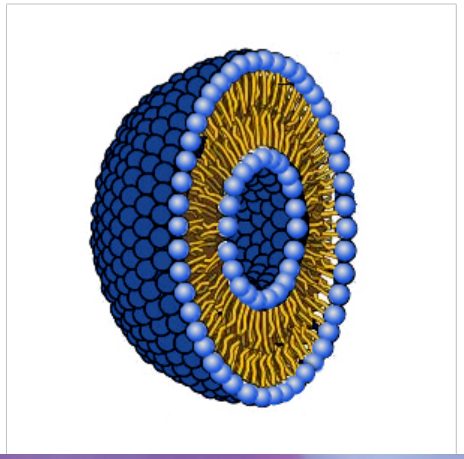
→ Infratherapeutic concentrations

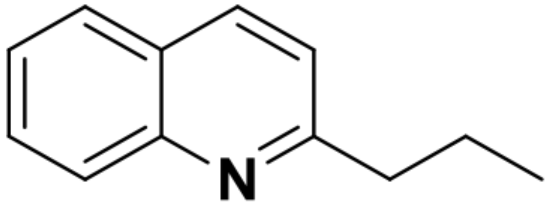
# Design of a 2-*n*-PQ liposomal formulation for intravenous route → visceral leishmaniasis

**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

| S. No | Egg PC (%) | Chol (%) | 2-PQ (%) | Size (nm) | EE (%) |
|---|---|---|---|---|---|
| 1 | 100 | --- | --- | 161 ± 2 | --- |
| 2 | 90 | 10 | --- | 172 ± 2 | --- |
| 3 | 80 | 20 | --- | 175 ± 2 | --- |
| 4 | 70 | 30 | --- | 182 ± 2 | --- |
| 5 | 95 | --- | 5 | 174 ± 2 | 41 |
| 6 | 90 | --- | 10 | 160 ± 3 | 7 |
| 7 | 80 | --- | 20 | 148 ± 3 | 13 |
| 8 | 85 | 10 | 5 | 148 ± 4 | 33 |
| 9 | 80 | 10 | 10 | 156 ± 3 | 53 |
| 10 | 70 | 10 | 20 | 164 ± 4 | 28 |
| 11 | 75 | 20 | 5 | 163 ± 4 | 47 |
| 12 | 70 | 20 | 10 | 158 ± 3 | 34 |
| 13 | 60 | 20 | 20 | 158 ± 3 | 30 |
| 14 | 65 | 30 | 5 | 146 ± 4 | 61 |
| 15 | 60 | 30 | 10 | 144 ± 2 | 5 |
| 16 | 50 | 30 | 20 | 158 ± 4 | 5 |

# *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

| Compound / Formulation | *In vitro* activity on *L. donovani* $IC_{50}$ (µM ± SD) | | | *In vivo* activity | | |
|---|---|---|---|---|---|---|
| | Axenic amastigotes | Intramacrophage amastigotes | Regimen | Number of mice | Route | Reduction of parasite burden (%) |
| Liposomal 2PQ | 3.10±0.25 | 5.84±0.31 | 3 mg/kg 2PQ x 5 days | 8 | iv | 83.8[a] |
| | | | 1.5 mg/kg 2PQ x 5 days | 8 | iv | 32.5[a] |
| | | | 0.75 mg/kg 2PQ x 5 days | 8 | iv | 5.2 |
| Liposomal 2PQ+AmB | 6.08±0.85 Eq 2PQ | 13.5±1.93 Eq 2PQ | 0.75 mg 2PQ + 0.006 mg AmB/kg x 5 | 8 | iv | 86.5[a] |
| | | | 0.37 mg 2PQ + 0.003 mg AmB/kg x 5 | 8 | iv | 10.3 |
| AmBisome® | 2.54±0.70 | 1.51±0.22 | 1 mg AmB/kg x 5 days | 8 | iv | 88.7[a] |
| | | | 0.25 mg AmB/kg x 5 days | 8 | iv | 27.1 |
| | | | 0.006 mg AmB/kg x 5 days | 8 | iv | 2.3 |
| Blank liposomes | Inactive | Inactive | Same suspension | 8 | iv | 5.7 |
| 2PQ | > 100 | >100 | / | / | / | / |
| Control (vehicle) | Inactive | Inactive | 0.2 mL x 5 days | 8 | iv | 0 |

[a] Significant versus control mice: $P<0.05$

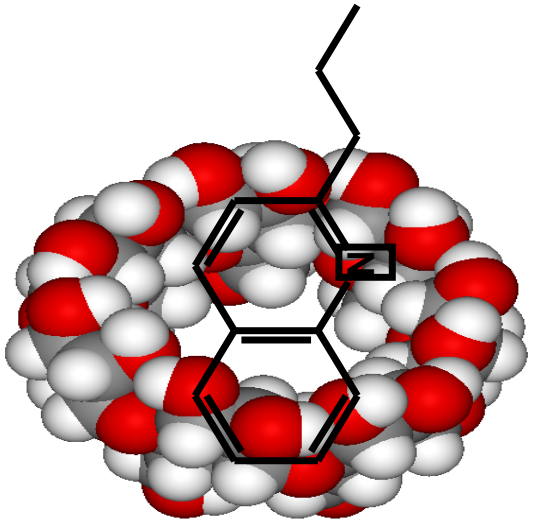
→ Liposomal 2-*n*-PQ: active at a total dose of 15 mg/kg

→ 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 → disseminated leishmaniasis

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

**Aim → Getting a hydroxypropyl-β-cyclodextrin (HPC) formulation → 2-*n*-PQ-HPC formulation**



→ Soluble enough for intravenous administration

→ Stable

→ Suitable for the treatment of experimental leishmaniasis

# *In vitro* activity of the 2-*n*-PQ-HPC formulation on *L. donovani*

| Compound/ formulation | *In vitro* activity on *L. donovani* $IC_{50}$ (µM ±SD)[a] | | Cytotoxicity Raw 264.7 MTC (µM ±SD) [b] | Selectivity Index (SI) SI= $MTC/IC_{50}$ [c] |
|---|---|---|---|---|
| | Axenic amastigotes | Intramacrophage amastigotes | | |
| 2-*n*-PQ | >100 | >100 | >100 | / |
| 2-*n*-PQ-HPC | 6.22±0.82 | 20.01±0.52 | >100 | >5 |
| HPC | >100 | >100 | >100 | / |
| Miltefosine | 1.22±0.50 | 0.85±0.21 | 50 | >50 |

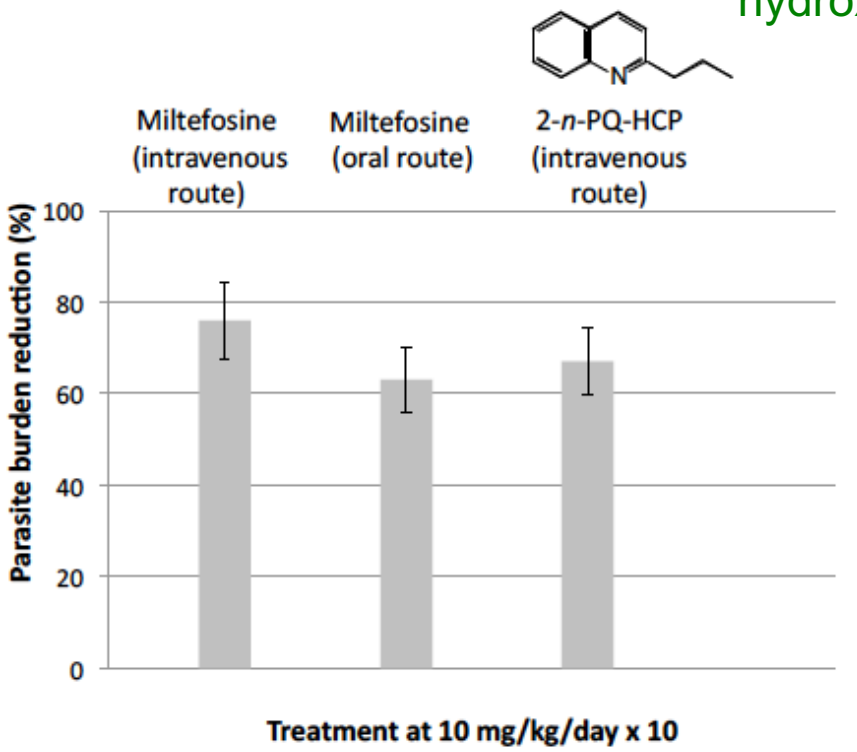
[a] Inhibitory Concentration 50% at 72 h, mean ± SD of three independent experiments
[b] Maximum Tolerated Concentration (MTC) at 72 h
[c] Selectivity Index (SI) calculated as the ratio of $MTC/IC_{50}$ on intramacrophage amastigotes

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

# *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)

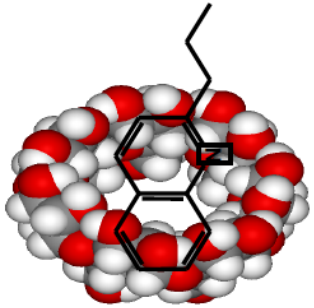
# Drug interactions and drug resistance of the 2-*n*-PQ-HPC formulation on *L. donovani*

→ 2-*n*-PQ-HPC exhibited similar activity on WT and drug-resistant parasites (Glu-R, AmB-R, Milt-R, Sita-R)

→ 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 = $IC_{50\ \text{after drug pressure}} / IC_{50\ \text{before drug pressure}}$



# Conclusion: from the plant to the formulations

From 2-*n*-PQ, a natural compound, easy to synthesize:

→1 liposomal formulation for intravenous route targeting VL
→ Active at 3 mg eq 2-*n*-PQ /kg /day x 5 days

→ 1 hydroxypropyl-β-cyclodextrin (HPC) formulation for intravenous route targeting disseminated leishmaniasis
→ Active at 10 mg eq 2-*n*-PQ /kg /day x 10 days

→ 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

DNDi
Drugs for Neglected Diseases *initiative*

DRUGABILIS
from molecules to drug candidates

CEFIPRA