Novel antimalarial enantiopure arylaminoalcohols as efflux pump substrates to fight resistant *P. falciparum*

Camille Tisnerat 1,2,*, Jérémy Schneider 1, Céline Damiani 1, Patrice Agnamey 1, Catherine Mullié 1, Anne Totet 1, Emmanuel Sevin 2, Alexandra Dassonville-Klimpt 1, Fabien Gosselet 2 and Pascal Sonnet 1

1 AGIR, UR4294, UFR de Pharmacie, Université de Picardie Jules Verne, Amiens, France
2 LBHE, UR2465, Université d'Artois, Lens, France

* Corresponding author: camille.tisnerat@etud.u-picardie.fr
Abstract

Malaria still is one of the most threatening diseases in the world. In 2019, the WHO estimated 229 million of cases and 409,000 deaths mainly due to the most prevalent and lethal *Plasmodium* species: *P. falciparum* (Pf).

Fighting resistant *Pf* strains is henceforth one of the main challenges to eradicate malaria. Indeed, parasites have developed resistances against all the available therapeutic arsenal, including artemisinin-based combination therapies (ACT). Studies of resistance phenotypes identified efflux pumps involved in this phenomenon of which the multidrug resistance ABC transporter *PfMDR1*. Its overexpression is partly responsible of the carrying of two ACT partner drugs, mefloquine (MQ) and lumefantrine (LM), into the food vacuole away from their cytosolic targets, leading to the efficacy decline of these arylaminoalcohol drugs.

In order to limit this efflux, our laboratory has developed efflux pump inhibitor (EPI) patterns based on previous described resistance reversing agents such as penfluridol. A library of novel arylaminoalcohols is easily affordable into a previously optimized synthesis to obtain MQ, LM and enpiroline analogs. This stereoselective and convergent synthesis requires a key arylvinyl converted to the corresponding enantiopure aryloxirane thanks to a Sharpless asymmetric dihydroxylation followed by a *one-pot* cyclization. Finally, a regioselective ring-opening by EPI moieties led to efflux pump substrate compounds.

Both design and synthesis of these arylaminoalcohols will be herein presented. *In vitro* efficacy against two *Pf* strains, cytotoxicity and preliminary results of P-gp, BCRP and MRPs efflux modulation in Caco-2 cells model will be reported. First structure-activity relationships will be discussed.

Keywords

Arylaminoalcohols ; asymmetric synthesis ; efflux pump modulation ; malaria ; *Plasmodium falciparum*
Malaria

229 million cases in 2019

409,000 deaths about 67% children under five

94% of all malaria deaths occur in Africa

Malaria

- **229 million** cases in 2019

- **409,000 deaths**

  - about **67% children** under five

- **94%** of all malaria deaths occur in **Africa**

Since the use of quinine, the failure of antimalarial therapies led to understand the molecular mechanisms behind drug resistance

Malaria

229 million cases in 2019

409,000 deaths

about 67% children under five

94% of all malaria deaths occur in Africa

Since the use of quinine, the failure of antimalarial therapies led to understand the molecular mechanisms behind drug resistance

Correlation between arylaminoalcohols (mefloquine – MQ, lumefantrine – LUM) and the transporter PfMDR1

Malaria

229 million cases in 2019

409,000 deaths

about 67% children under five

94% of all malaria deaths occur in Africa

Since the use of quinine, the failure of antimalarial therapies led to understand the molecular mechanisms behind drug resistance

Correlation between arylaminoalcohols (mefloquine – MQ, lumefantrine – LUM) and the transporter PfMDR1

Malaria

229 million cases in 2019

409,000 deaths

about 67% children under five

94% of all malaria deaths

occur in Africa

Since the use of quinine, the failure of antimalarial therapies led to understand the molecular mechanisms behind drug resistance

Correlation between arylaminoalcohols (mefloquine – MQ, lumefantrine – LUM) and the transporter PfMDR1

How to limit this efflux?

MQ cannot reach its cytosolic target

Overexpression and/or mutation of PfMDR1


The 7th International Electronic Conference on Medicinal Chemistry
01–30 November 2021 | ONLINE
Malaria

229 million cases in 2019

409,000 deaths
about 67% children under five

94% of all malaria deaths occur in Africa

Since the use of quinine, the failure of antimalarial therapies led to understand the molecular mechanisms behind drug resistance

Correlation between arylaminoalcohols (mefloquine – MQ, lumefantrine – LUM) and the transporter PfMDR1

How to limit this efflux?

Hybrids strategy
Efflux pump inhibitor (EPI) moiety coupled with arylaminoalcohol pattern to block PfMDR1

Design and synthesis

Known hybrids in CQ-R strains

\[ \text{IC}_{50} \text{ (PfDd2)} = 5.3 \text{ nM} \]

\[ \text{IC}_{50} \text{ (PfDd2)} = 1.8 \text{ nM} \]

\[ \text{IC}_{50} \text{ (PfDd2)} = 2.6 \text{ nM} \]

\[ \text{IC}_{50} \text{ (PfDd2)} = 4.2 \text{ nM} \]

*J. Med. Chem. (2010), 53, 6477–6489*
Design and synthesis

In MQ-R strains, shift in sensitivity with penfluridol

**MQ alone**

IC$_{50}$ (PfD6) = 33.7 nM

**MQ + penfluridol (0.5 µM)**

IC$_{50}$ (PfD6) = 13.7 nM


Known hybrids in CQ-R strains

**IC$_{50}$ (PfDd2)**

R = H, IC$_{50}$ (PfDd2) = 2.6 nM

R = Ph, IC$_{50}$ (PfDd2) = 4.2 nM

Design and synthesis

In MQ-R strains, shift in sensitivity with penfluridol

- MQ alone
  \[ IC_{50} \text{ (PfD6)} = 33.7 \text{ nM} \]

- MQ + penfluridol (0.5 µM)
  \[ IC_{50} \text{ (PfD6)} = 13.7 \text{ nM} \]

\[ \text{Trans. R. Soc. Trop. Med. Hyg. (1993), 87, 81-83} \]

Known hybrids in CQ-R strains

\[ \text{J. Med. Chem. (2010), 53, 6477–6489} \]

\[ \text{IC}_{50} \text{ (PfDd2)} = 5.3 \text{ nM} \]

\[ R = H, \text{ IC}_{50} \text{ (PfDd2)} = 2.6 \text{ nM} \]

\[ R = \text{Ph}, \text{ IC}_{50} \text{ (PfDd2)} = 4.2 \text{ nM} \]
**Design and synthesis**

In MQ-R strains, shift in sensitivity with penfluridol

- **MQ alone**
  - $IC_{50} (PfD6) = 33.7$ nM

- **MQ + penfluridol (0.5 µM)**
  - $IC_{50} (PfD6) = 13.7$ nM

*Trans. R. Soc. Trop. Med. Hyg. (1993), 87, 81-83*

**Known hybrids in CQ-R strains**

- **CQ + imipramine**
  - $IC_{50} (PfDd2) = 5.3$ nM

- **CQ + Ad**
  - $IC_{50} (PfDd2) = 1.8$ nM

- **CQ + R = H**
  - $IC_{50} (PfDd2) = 2.6$ nM

- **CQ + R = Ph**
  - $IC_{50} (PfDd2) = 4.2$ nM

*J. Med. Chem. (2010), 53, 6477–6489*

---

**Chemical Structures**

- **MQ**
  - ![Chemical structure of MQ](image1)
- **Penfluridol**
  - ![Chemical structure of Penfluridol](image2)

---

**Synthesis Diagram**

- **Imipramine Synthesis**
  - ![Imipramine synthesis](image3)

- **CQ + Ad Synthesis**
  - ![CQ + Ad synthesis](image4)

- **CQ + R = H Synthesis**
  - ![CQ + R = H synthesis](image5)

- **CQ + R = Ph Synthesis**
  - ![CQ + R = Ph synthesis](image6)
Biological properties

1 Nanomolar range activity

\[ IC_{50} (\text{mM}) \]

<table>
<thead>
<tr>
<th></th>
<th>Pf3D7</th>
<th>PfW2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-R</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>1-S</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>2-R</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>2-S</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>3-R</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>3-S</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>4-R</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>4-S</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>5-R</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>5-S</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>6-R</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>6-S</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Pf3D7 = MQ attenuated sensitivity; PfW2 = CQ-R
Biological properties

1. Nanomolar range activity

- Pf3D7 = MQ attenuated sensitivity; PfW2 = CQ-R

2. High selectivity index in quinoline series (> 450)
Modulation of PfMDR1

3 No MRPs modulation
4 Pgp/BCRP modulation

Modulation of *PfMDR1*

3. No MRPs modulation
4. Pgp/BCRP modulation

Modulation of PfMDR1

3 No MRPs modulation
4 Pgp/BCRP modulation


![Chemical structures of drugs](image)
Modulation of *PfMDR1*

3. No MRPs modulation
4. Pgp/BCRP modulation


Except 2 and 6, modulation at 10 µM
Modulation of PfMDR1

3 No MRPs modulation
4 Pgp/BCRP modulation


Except 2 and 6, modulation at 10 µM

![Graph showing modulation of compounds](image)
Conclusion & Prospects

3. No MRPs modulation
4. Pgp/BCRP modulation

A miniaturized pump out method for characterizing molecule interaction with ABC transporters: *Int. J. Mol. Sci. (2019)*, **20** (22), 5529

Except 2 and 6, modulation at 10 µM

Support our strategy of hybrids synthesis

Next step: antimalarial activity against Dd2 strains
Acknowledgements