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Enantiopure aminoaryl-alcohols with fluorene core and their antimalarial activities

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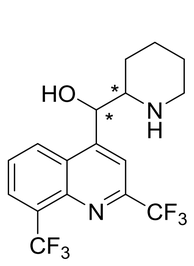
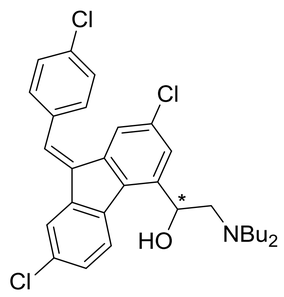
INTRODUCTION

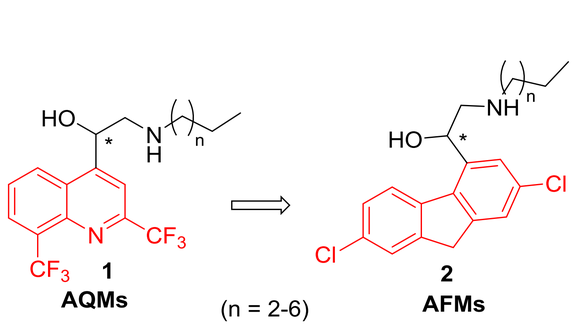
Malaria, the world's most deadly parasitic disease, is caused by ***Plasmodium*** species which are transmitted by female *Anopheles* mosquitoes. In 2015, 210 million cases of malaria and almost 430000 deaths due to the parasite[a], in particular by ***P. falciparum***, were listed. In drug resistance areas, recommended by WHO since 2001, several antimalarial drugs, such as **mefloquine** (MQ) or **lumefantrine** (LM), are currently used in combination with artemisinin derivatives (ACTs).[a,b] However, the emergence of multi-drug-resistant parasites, including artemisinin, is a real problem of public health. We have previously developed an asymmetric synthesis to prepare enantiopure 4-aminoquinoline-methanols (**AQMs**) as **MQ** analogs. They were active in the nanomolar range against 3D7 (chloroquine-sensitive) and W2 (chloroquine-resistant) *P. falciparum* strains (Table 1).[c] Interestingly, *(S)*-enantiomers displayed an activity increased by 2 to 15-fold as compared to their *(R)*-counterparts.[c,d] In order to obtain new antimalarial agents and evaluate the heterocyclic modification (fluorene *vs* quinoline) on the antimalarial activity, we describe here, the syntheses of enantiopurs 4-aminofluorene-methanols (**AFMs**) as LM analogs. We will establish novel structure-activity relationships (SAR), with several structural modifications, and we will compare the **AFMs** antimalarial activities with the **AQMs** previously obtained.



Mefloquine (MQ)



Lumefantrine (LM)



1 AQMs (n = 2-6) **2 AFMs**

Table 1: Antimalarial activity of **AQMs** 1 with hypoxanthine ^{3}H based method[c]

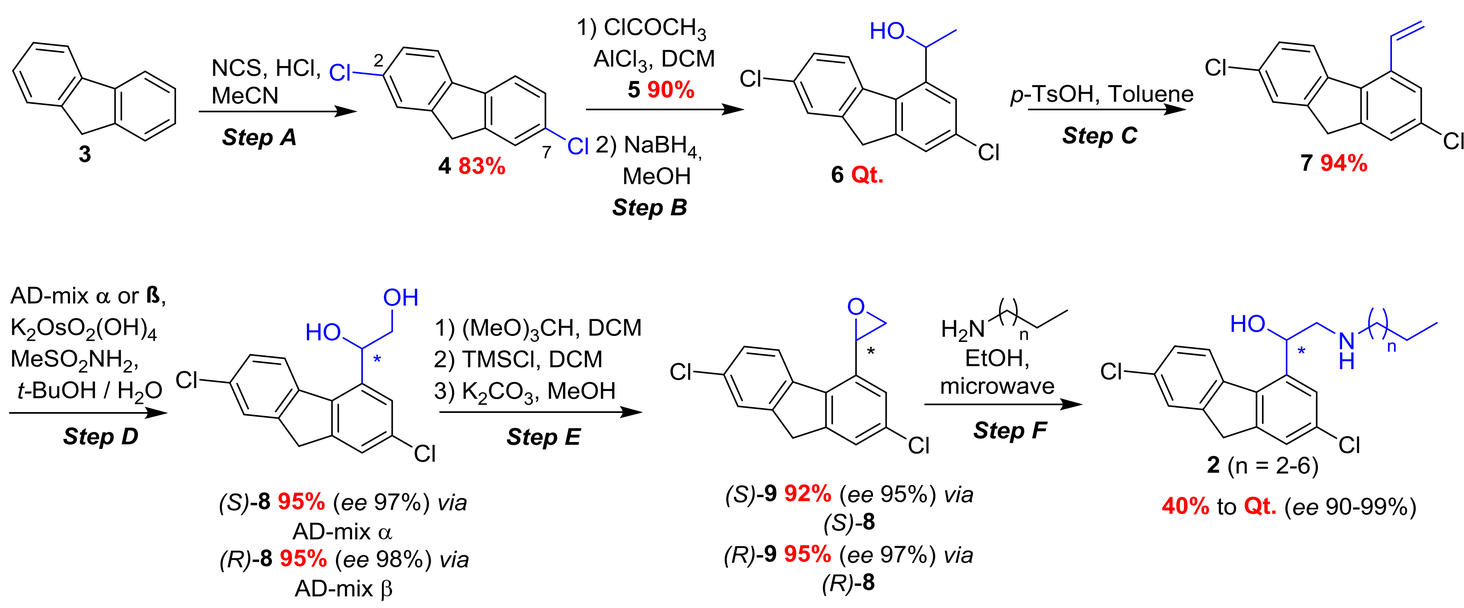
Product	IC_{50} (nM)	
	***Pf*3D7**	***Pf*W2**
MQ	52.2 ± 16.3	10.4 ± 2.4
CQ	18.9 ± 0.1	572 ± 0.1
(*S*)-**1a** (n=3)	8.3 ±0.4	6.9 ± 0.6
(*R*)-**1a** (n=3)	74.7 ± 4.7	38,2 ± 3.6
(*S*)-**1b** (n=4)	205 ± 16.2	142 ± 13.8
(*R*)-**1b** (n=4)	14.5 ± 1.2	9.4 ± 0.9
(*S*)-**1c** (n=5)	33.0 ± 1.6	ND[1]
(*R*)-**1c** (n=5)	254 ± 27	ND[1]

1. Not determined

ENANTIOSELECTIVE AFMs SYNTHESIS

To obtain the enantiopure **AFMs**, the first step was a chloration from commercial fluorene **3** in position two and seven (**Step A**). Then a Friedel-Craft acylation and reduction give the compound **6**. The vinyl **7** was prepared *via* **6** dehydratation (Step C). Then, an asymmetric Sharpless dihydroxylation was performed to give the diols (*S*)-**8** and (*R*)-**8** in 95% yields (*ee* > 97%) (Step D). The epoxides **9** were obtained after a one-pot synthesis (in three reaction steps) in good yields and enantiomeric purities superior to 95% (Step E). A nucleophilic addition, by microwave assistance, in the presence of a primary amine gave the enantiopure **AFMs 2** (Step F).

Currently, we obtained 10 **AFMs 2** with good yields and very good enantiomeric excess (*ee* > 98%).



RESULTS

Relative antimalarial activity indexes

For *Pf*3D7 : IC_{50} MQ (nM) / IC_{50} compound (nM)
For *Pf*W2 : IC_{50} CQ (nM) / IC_{50} compound(nM)

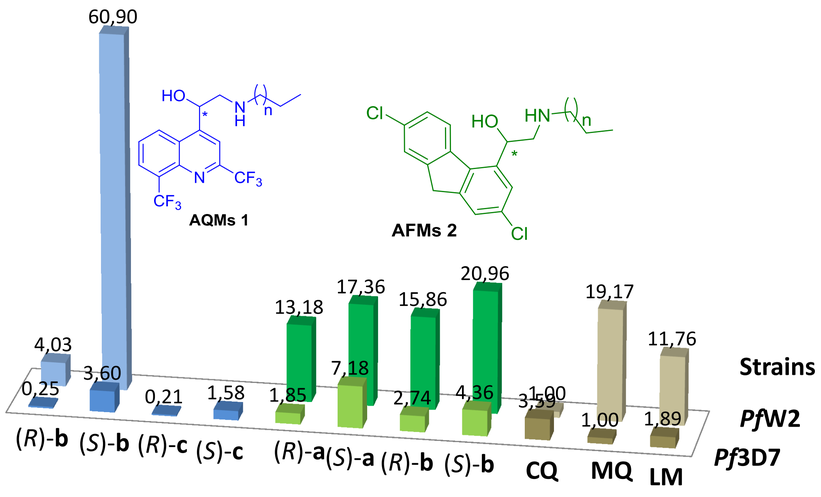


Table 2: Antimalarial activities of **AFMs 2a,b** with ELISA HRP2 method.

Product	IC_{50} (nM)	
	***Pf*3D7**	***Pf* W2**
MQ	61.0 ± 7.1	21.1 ± 5.0
LM	28.7 ± 3.5	39.9 ± 2.8
CQ	17.0 ± 4.6	404.5 ± 55.2
(*R*)-**2a** (n = 3)	33.0 ± 6.2	30.7 ± 3.3
(*S*)-**2a** (n = 3)	8.5 ± 1.8	23.3 ± 4.5
(*R*)-**2b** (n = 4)	22.3 ± 2.5	25.5 ± 3.9
(*S*)- **2b** (n = 4)	14.0 ± 2.7	19.3 ± 4.8

Table 3: **AFMs** cytotoxicity on HepG2

Product	IC_{50} (µM)	Selectivity indexes[2]
	HepG2	
MQ	11.9 ± 6.9	195
(*R*)-**2a**	4.82 ± 1.0	142
(*R*)-**2b**	6.7 ± 4.5	3164
(*S*)-**2b**	5.0 ± 1.6	520

2 IC_{50} HePG2 / IC_{50} compound on *Pf*3D7

The *in vitro* antimalarial activities of **AFMs 2** were evaluated against 3D7 and W2 *P. falciparum* strains with ELISA HRP2 method. The hypoxanthine method was previously used to assess the **AQMs 1** antimalarial activities. To be able to compare the **AQMs 1** and **AFMs 2**, as these two methods were different, relative antimalarial activity indexes were calculated and compared respectively to MQ (for 3D7) and to CQ (for W2).

The **AFMs** were active on the nanomolar range with a slightly higher efficacy against 3D7 strains for (*S*)-enantiomers (Table 2). The (*S*)-**AFMs 2a** and **2b** had activities similar to their analogues (*S*)-**AQMs 1b** and **1c**. Interestingly, the (*S*)-**AFMs** displayed an activity increased by 1.6 to 3.9-fold as compared to their *(R)*-counterparts. So, a fluorene is a promising core for antimalarial activity. It is beneficial for the corresponding (*R*)-enantiomers. The (*S*)-compounds were generally more actives that **LM**, **MQ** and **CQ** on 3D7 and W2. The **AFMs** were not cytotoxic on HepG2 cells (a human liver cancer cell line) with selectivity indexes superiors to 100 (Table 3).

CONCLUSION

In this work, an asymmetric route to access the **AFMs** was developed. We obtained 10 **AFMs**, in 7 steps, with 17% to 56% of global yields and enantiomeric excess superior to 90%. Preliminary antimalarial results for **AFM 2a** and **AFM 2b** showed high activities on the nanomolar range that confirm the interest of the fluorene core in the research of new antimalarial drugs. The importance of the stereochemistry was showed with high activity for (*S*)-enantiomers. The **AFMs** presented a low cytotoxicity *in vitro* on HepG2. In the continuation of our work, we will complete their toxicity and *in vivo* evaluations.

(a) World Malaria Report, *WHO*, **2015**, 2-81. - (b) Guidelines for the treatment of malaria, 3RD edition, *WHO*, **2015**, 214 -219. – (c) Jonet A. *et al.* , Tetrahedron: Asymmetry, 22, **2011**, 138-148 . - (d) Mullié C. *et al., Malaria Journal*, **2012**, 11 : 65.