Novel Racemic and Enantiopure Amino-Fluorene-Methanol Compounds with Antimalarial Activities

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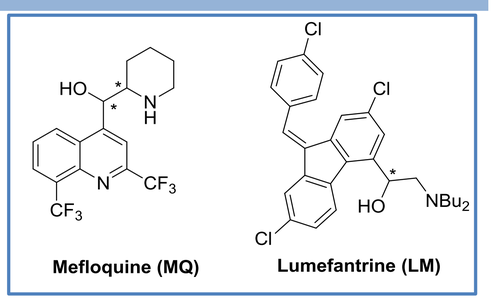
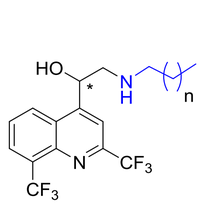
INTRODUCTION

Malaria, the world's most deadly parasitic disease, is caused by *Plasmodium* species which are transmitted by female *Anopheles* mosquitoes. In 2015, 214 million cases of malaria and 438 000 deaths due to the parasite[a], in particular by *P. falciparum*, were listed. In drugs resistance areas, several antimalarial drug, such as mefloquine (MQ) or lumefantrine (LM), are currently used in combination with artemisinin derivatives (ACTs). These ACTs are recommended by WHO since 2001[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 enantiopur 4-aminoquinoline-methanols (AQMs) as MQ analogs. They were active on 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[d,e]. In order to obtain new antimalarial agents with novel aromatic core, we describe here, an enantioselective synthesis of 4-aminofluorene-methanols (AFMs) as LM analogs. We will establish novel structure-activity relationships (SAR) and we will compare the activities with the AQMs **1** previously obtained.





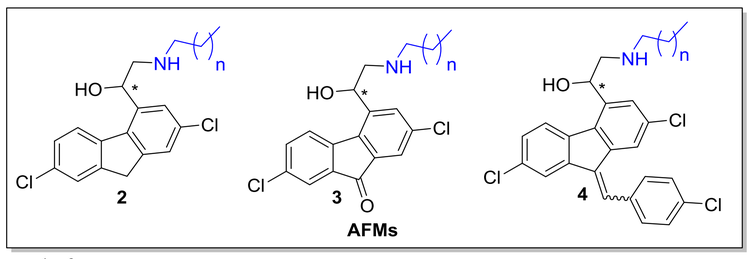


Table 1: AQM IC_{50} (nM) [c]

	Product	MQ	CQ	(*S*)-1a (n=3)	(*R*)-1a (n=3)	(*S*)-1b (n=5)	(*R*)-1b (n=5)
IC_{50}[a] (nM)	*P. falciparum* 3D7	52,2 ± 16,29	18,9 ± 0,08	8,33 ±0,44	74,7 ± 4,7	33,0 ± 1,6	254 ± 27
	P. falciparum W2	10,4 ± 2,42	572 ± 0,09	6,98 ± 0,62	38,2 ± 3,6	ND[b]	ND[b]

[a] Isotope micromethod that measures inhibition of parasite uptake of tritiated hypoxanthine in the presence of antimalarial agents [b]. Not determined

RACEMIC AND ENANTIOSELECTIVE AFMs SYNTHESIS

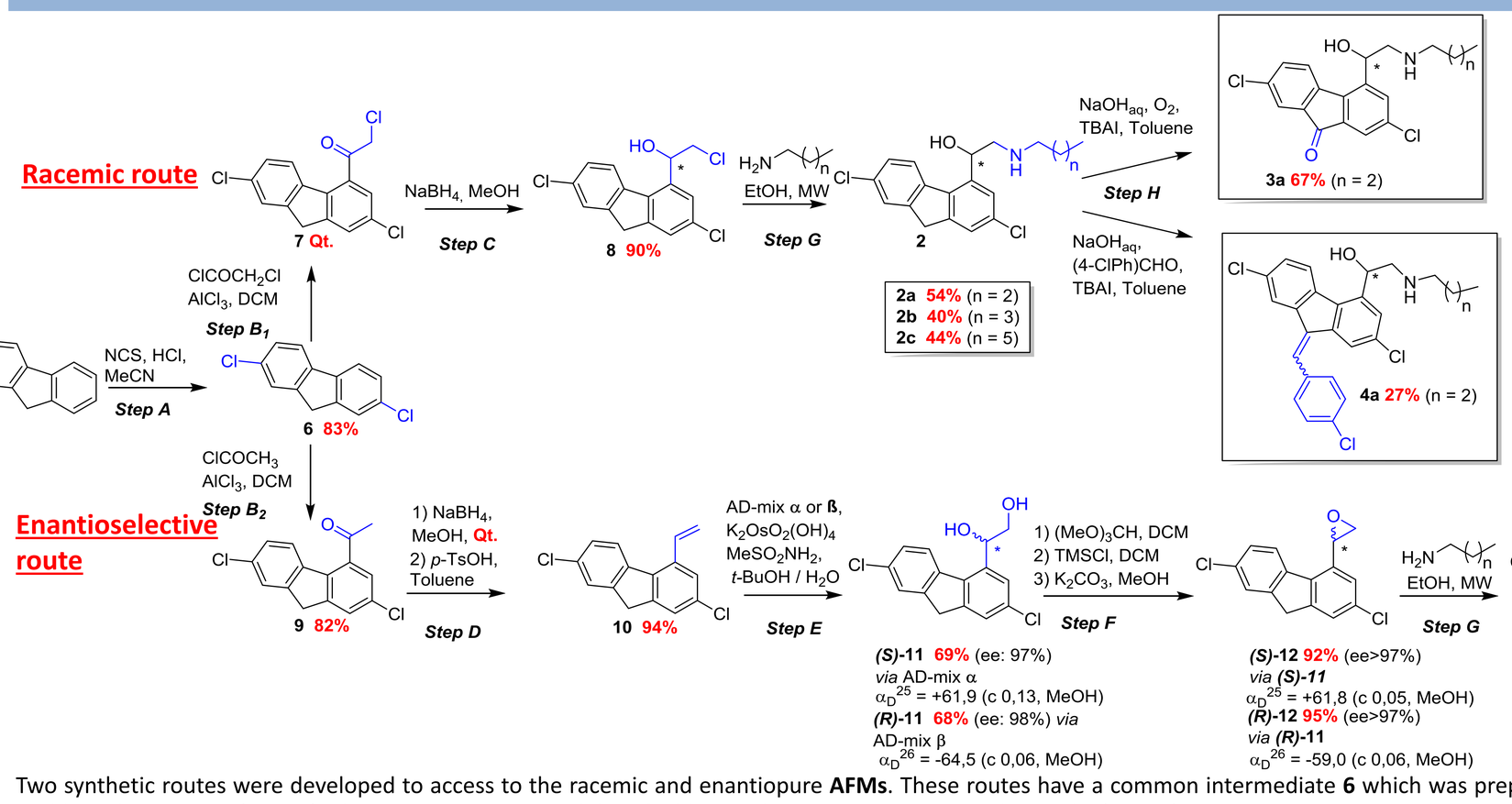


Table 2: AFMs 2a-d (Step G)

AFM	n	Yield (%)	ee. (%)[a]
(*R*)-2a	2	78	99
(*S*)-2a	2	30	98
(*R*)-2b	3	79	98
(*S*)-2b	3	46	99
(*S*)-2c	4	56	99
(*R*)-2c	4	Qt.	99
(*R*)-2d	5	83	99
(*S*)-2d	5	60	90
(*R*)-2e	6	48	99
(S)-2e	6	56	98

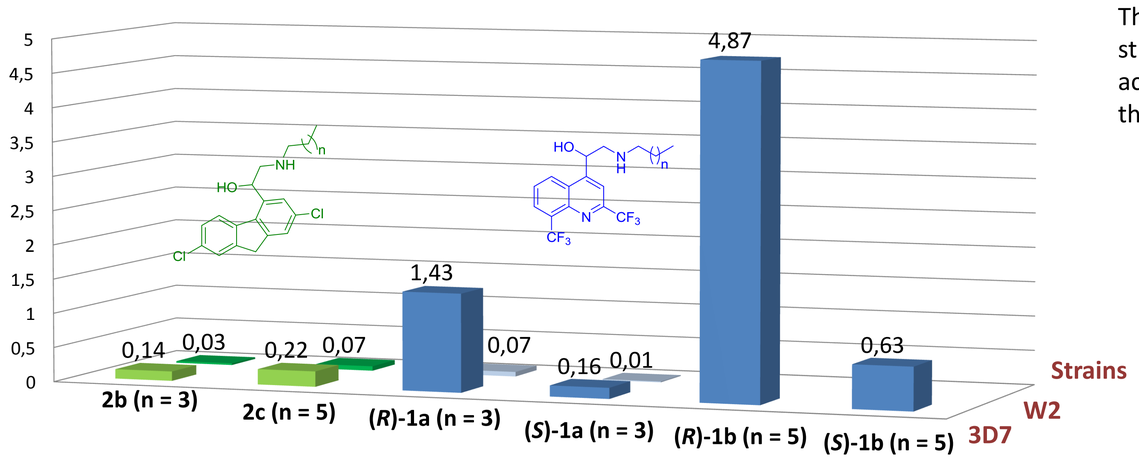
[a] determined with chiral HPLC

Two synthetic routes were developed to access to the racemic and enantiopure **AFMs**. These routes have a common intermediate **6** which was prepared from available commercially fluorene **5** through a radical Wohl-Ziegler reaction (Step A). The next step was a Friedel-Craft chloroacylation to give compounds **7** and **9** (Step B).
In first time, to have a quick access to AFMs and study their antimalarial activities, we have developed a racemic route. For this, the chlorhydrine **8** was synthesized with 90% yield (Step C). The racemic AFMs **2a-c** were obtained in the presence of a primary amine by microwave assistance. Then, an oxidation or a Knoevenagel condensation gave respectively the racemic AFMs **3a** and **4a**.
To obtain the enantiopure AFMs, we chose to develop an asymmetric epoxidation. The vinyl **10** was prepared *via* **9** reduction followed by dehydratation. Then, an asymmetric Sharpless dihydroxylation was performed to give the diols **(*S*)-11** and **(*R*)-11** with 68% and 69% of yields (ee. >97%) (Step E). The epoxides **12** were obtained after a one-pot synthesis (in three reaction steps) in good yields and enantiomeric purities (Step F). A nucleophilic addition, by microwave assistance in the presence of a primary amine gave the enantiopure **AFMs 2a-d** (Step G, Table 2). Currently, we obtained many enantiopure (ee.>98%) **AFMs 2a-d** with good yields (Table 2).

RESULTS

Relative antimalarial activity indexes

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



The *in vitro* antimalarial activity of **AFMs 2** were evaluated against 3D7 and W2 *P. falciparum* strains with a SYBR Green I fluorescence-based method. The hypoxanthine method was previously used to assess the **AQMs 1** antimalarial activity. 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 racemic AFMs **2b-d** were active on the nanomolar range with a slightly higher efficiency against W2 strains (Table 3). In comparison, we can see that **2b, c** showed similar activities with the **(*S*)-AQMs** (the most active MQ analogs). These first results support us for the synthesis of new enantiopure LM analogs to study the influence of the stereochemistry.

Table 3: IC_{50} (nM) AFMs

	Product	MQ	CQ	2b	2c	2d
IC_{50}[a] (nM)	*P. falciparum* 3D7	131,5 ± 16,29	29,6 ± 0,59	18,1 ± 1,65	29,4 ± 6,91	<39
	P. falciparum W2	10,4 ± 2,42	284,3 ± 32,16	7,1 ± 0,18	18,6 ± 1,88	<39

[a] SYBR Green I *in vitro* test was used

CONCLUSION

In this work, two routes to access to racemic and enantiopure AFMs **2, 3** and **4** were investigated. At this time, we obtained 5 racemic and 10 enantiopure **AFMs,** in 5 or 7 steps, with 14% to 37% of global yields. Preliminary antimalarial results for **AFM 2b, AFM 2c** and **AFM 2d** showed high activities on the nanomolar range that confirm the interest of the flurorene core in the research of new antimalarial drugs. We must now verify the importance of an asymmetric center by biological evaluation of enantiopure **AFMs** and continue the fluorene core pharmacomodulations.

(a) World Malaria Report, *WHO*, **2015**, 2 - 81. - (b) 1-Guidelines for the treatment of malaria, 3RD edition, *WHO*, **2015**, 214 - 219. – (c) Petersen I. *et al*., *FEBS LETTERS 585*, **2011**, 15, 515-562. - (d) Jonet A. et al. , Tetrahedron: Asymmetry, 22, **2011**, 138–148 . - (e) Mullié C. *et al., Malaria Journal*, **2012**, 11 : 65.