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Synthesis of New Enantiopure Aminoalcohol Fluorenes as Promising Antimalarial Compounds

Camille Tisnerat, [a] Jérémy Schneider, [a] Romain Mustière, [a] Aurélie Herrero, [a] René Momha, [a] Céline Damiani, [a] Patrice Agnamey, [a] Anne Totet, [a] Mathieu Marchivie, [b] Jean Guillon, [c] Alexandra Dassonville-Klimpt, [a]* Pascal Sonnet [a]*

[a] Dr. C. Tisnerat (CT), Dr. J. Schneider (JS), Dr. R. Mustière (RMu), Dr. R. Momha (RMo), A. Herrero (AH), Dr. C. Damiani (CD), Pr. P. Agnamey (PA), Pr. A. Totet (AT), Pr. A. Dassonville-Klimpt (ADK), Pr. P. Sonnet (PS)
AGIR, UR 4294
UFR de Pharmacie, Université de Picardie Jules Verne, Amiens, France
E-mail: alexandra.dassonville-klimpt@u-picardie.fr (ADK); pascal.sonnet@u-picardie.fr (PS).

[b] Dr. M. Marchivie (MM)
Université de Bordeaux, CNRS, Bordeaux, INP, ICMCB, UMR 5026, Pessac F-33600, France.

[c] Pr. J. Guillon (JG)
INSERM U1212, UMR CNRS 5320, Laboratoire ARNA
UFR des Sciences Pharmaceutiques, Université de Bordeaux, Bordeaux, France

Abstract

Herein, we report the design, synthesis, and characterisation of a new library of enantiopure aminoalcohol fluorenes, as well as their *in vitro* evaluation for biological properties, including activity against two strains of *P. falciparum* (3D7 and W2) and cytotoxicity on the HepG2 cell line. All tested compounds exhibited good to excellent antimalarial potency with IC_{50} values ranging from 0.7 to 70.2 nM whatever the strain. Interestingly, most compounds showed equal or better antimalarial activity compared to the reference drugs lumefantrine, mefloquine and chloroquine. Despite moderate cytotoxicity in the micromolar range, all aminoalcohol fluorenes displayed an excellent selectivity index higher than 100 due to strong antimalarial activity. Furthermore, we report *in silico* analyses of physicochemical and pharmacokinetic properties for all compounds, highlighting the drug-likeness of compound **10** and its promising potential for further studies.

Introduction

Malaria remains a significant public health challenge, with the World Health Organization (WHO) reporting 249 million malaria cases and 608,000 deaths in 2022.[1] Africa supports the heaviest burden, accounting for 82% of cases and 94% of deaths. Most infections are caused by the *Plasmodium falciparum* (*Pf*) parasite. Despite progresses in controlling the disease, the global spread of *P. falciparum* strains resistant to nearly all available antimalarial drugs, including artemisinin-based combination therapies (ACT), threatens efforts to control malaria.[2–5] Consequently, there is an urgent need for the development of new effective antimalarial drugs.

One achievable strategy is to optimise current families of antimalarial drugs in order to improve their efficacy (activity, toxicity and pharmacokinetics) or to circumvent the resistance mechanisms that undermine their effectiveness. Our research group is focused on arylaminoalcohols, a historically significant antimalarial family still used in ACT, such as mefloquine (MQ) and lumefantrine (LM), despite their undefined mode of action.[6–10] MQ is used in combination with dihydroartemisinin but is known to have side effects limiting its use.[11,12] Moreover, the antimalarial efficacy is compromised by the *Pf* multidrug resistance 1 (*Pf*MDR1) protein responsible for drug resistance.[13–19] In combination with artemether, LM is the only marketed drug without reported resistance markers but it requires administration with a fatty meal to improve absorption due to its high lipophilicity.[20–22] Therefore, the development of new arylaminoalcohols with enhanced efficacy against resistant strains, alongside with improved drug-likeness properties, is crucial to optimise antimalarial therapies.

We have reported the asymmetric synthesis of such compounds based on various bioisostere aryl core, including quinoline, pyrrolo[1,2-*a*]quinoxaline and 6-arylpyridine.[23–33] Excellent nanomolar antimalarial activity of both quinoline such as ((*S*)-**1**) and 6-arylpyridine series containing an alkyl chain against *Pf* strains with different resistance profile were reported.[26,32] Interestingly, the selectivity favouring one enantiomer over the other confirmed our asymmetric synthesis strategy. Recent structure-activity relationship (SAR) studies on new aminoalcohol quinolines highlighted that alkyl chain modifications, including the addition of fluorine atoms, a second stereogenic center ((*S*)-**2**), or a potential efflux pump inhibitor (EPI) moiety, were well-tolerated. These potential EPI are often composed of two aromatic hydrophobic groups and a hydrogen bond acceptor site, preferably on a side-chain nitrogen atom. We have recently described the strong antimalarial activity of hybrids with 2,8-bis(trifluoromethyl)quinoline core and a diphenylmethyl moiety as potential EPI such as ((*S*)-**3**).[33] The three antimalarial lead compounds, (*S*)-**1**, (*S*)-**2** and (*S*)-**3** were characterised by potent antimalarial activity (IC_{50} = 8-36 nM), moderate cytotoxicity (6-14 μM) and good selectivity index (> 100) (**Figure 1**). In

parallel, pharmacokinetic studies indicated that metabolic stability could be improved, particularly for those with a long alkyl chain (*S*)-**1** or a 1-methoxy-3-phenylpropan-2-yl group (*S*)-**2**.

Inspired by these promising results, we describe here a further study on arylaminoalcohols using LM's fluorene scaffold. Exploration of this structure remains underrepresented in the literature,[34] whereas reducing lipophilicity could improve physicochemical and pharmacokinetic properties while maintaining good activity. Hence, we developed simplified LM analogues by removing the substitution at the C9-position in order to reduce the lipophilicity and shorten the synthesis. The growing trend towards the development of enantiomerically pure drugs and the eudysmic ratio observed in previous quinoline and 6-arylpyridine series prompted us again to consider asymmetric synthesis early in our study, along with racemic synthesis.[26,32,35] The fluorene core was combined with amino chains from our most active aminoalcohol quinolines. New amino chains, containing fluorine and bulky groups to counter potential metabolisation, were also considered.

Here, we report the racemic and asymmetric synthesis of fluorene analogues, as well as their *in vitro* antimalarial activity and cytotoxicity. The prediction of drug-likeness behaviour based on *in silico* analyses of physicochemical and pharmacokinetic properties is also discussed.

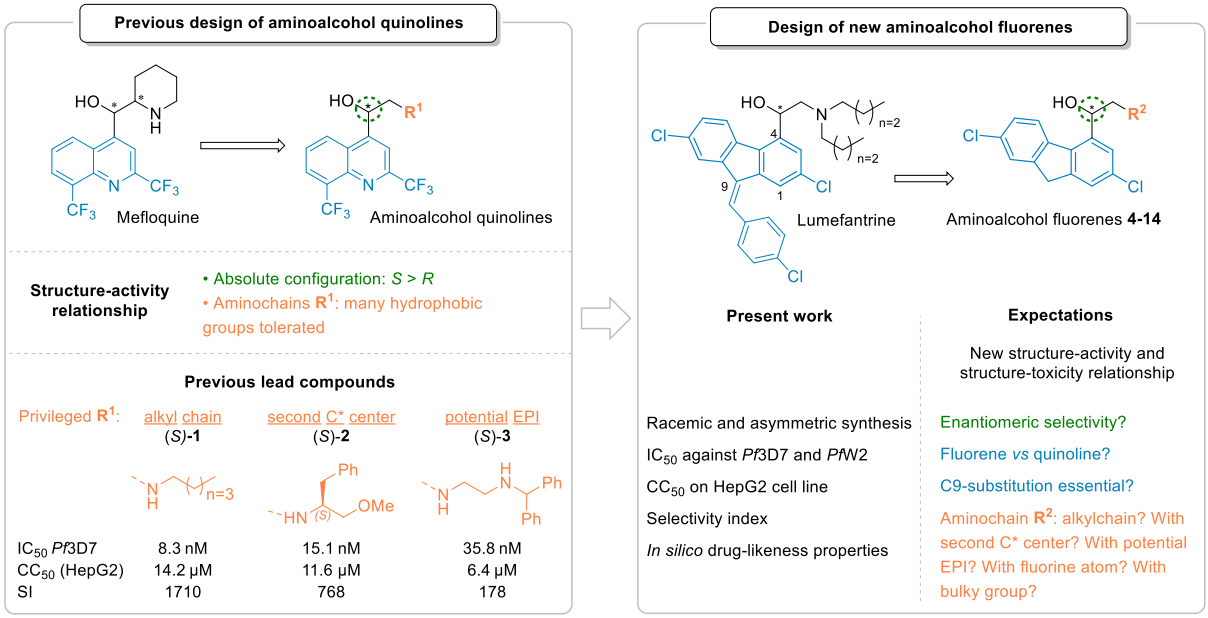
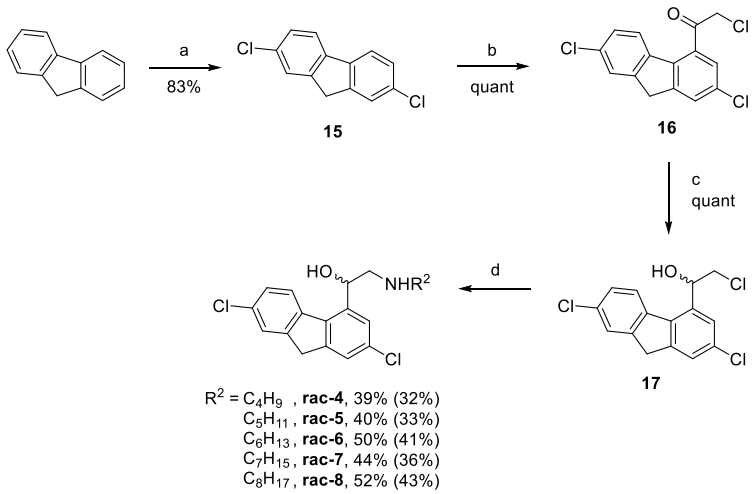


Figure 1. Previous work and design of new antimalarial aminoalcohol fluorenes **4**-**14**.

Results and discussion

Chemistry

The racemic synthesis of the aminoalcohol fluorenes **rac-4-8** is illustrated in **Scheme 1**. Commercial fluorene was selectively chlorinated at the C2 and C7 positions to afford **15** in 83% yield, using *in situ* generation of gaseous chloride from *N*-chlorosuccinimide and hydrochloric acid.[36] Then, a Friedel-Craft acylation was carried out to obtain the acyl derivative **16**, which was quantitatively reduced to lead to the racemic chlorohydrin **17**. Finally, nucleophilic substitution with the desired alkylamine yielded the final compounds. The five racemic aminoalcohol fluorenes **rac-4-8**, with alkyl chains ranging from 4 to 8 carbons, were synthesised in moderate yields (32-43%) over four steps.



Scheme 1. Racemic route for the aminoalcohol fluorenes. Reagents and conditions: (a) NCS, HCl_{aq} 37%, MeCN, 0-20°C. (b) $ClCOCH_2Cl$, $AlCl_3$, CH_2Cl_2, 0-20°C. (c) $NaBH_4$, MeOH, 0-20°C. (d) R^2NH_2, EtOH. The overall yields are indicated in bracket.

Initially, we attempted to enantioselectively reduce ketone **16** into the corresponding enantiopure chlorohydrins **17** to access the enantiomers of compounds **4-8**. However, despite extensive efforts to optimise the reaction, including variations in the nature and quantity of both the reductive agent and the chiral ligand, as well as changes in reaction conditions (temperature, solvent, and concentration), the results were disappointing in terms of enantioselectivity and/or yield. The best result was obtained using 1.5 equivalent of $LiAlH_4$ as reducing agent, the (*S*)-BINOL as chiral ligand (2 equivalents) in THF at -78°C, where the chlorohydrin (*R*)-**17** was afforded with 75% enantiomeric excess (*ee*) and 50% yield (data not shown).

Consequently, we focused on our previously described and successful indirect asymmetric epoxidation strategy, which involved obtaining a key vinyl intermediate (**Scheme 2**).[24,29,32,37] Starting from dichlorofluorene **15**, Friedel-Craft acylation with acetyl chloride afforded ketone **18**, which was then reduced to the corresponding alcohol **19** using sodium borohydride.

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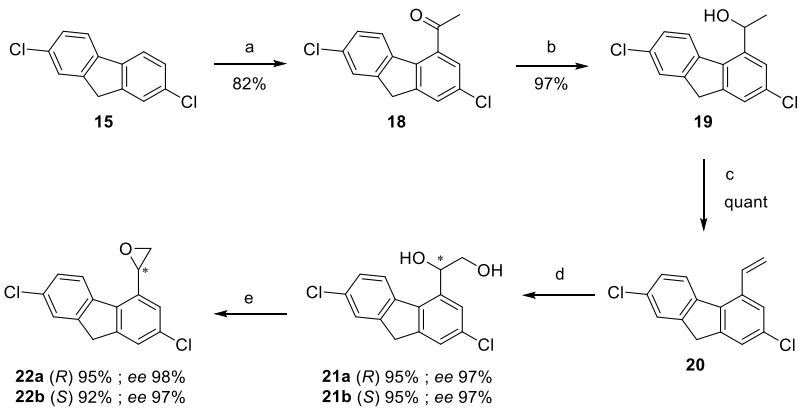
Subsequent dehydration under acidic conditions with heating, performed using a distilling trap, quantitatively led to the key vinyl **20**, which was synthesised in 79% overall yield over three steps. Sharpless dihydroxylation, using AD-mix α or β supplemented with potassium osmate and methanesulfonamide, gave diols **21a** (*R*) and **21b** (*S*) with excellent *ee* (97%) and yield (95%). A one-pot ring closure, retaining the absolute configuration, was then carried out to form the corresponding enantiopure epoxides **22a** (*R*) and **22b** (*S*) with 95% and 92% yield respectively.



Scheme 2. Asymmetric route for enantiopure aminoalcohol fluorenes. Reagents and conditions: (a) CH_3COCl, $AlCl_3$, CH_2Cl_2, 0-20°C. (b) $NaBH_4$, MeOH, 0-20°C. (c) PTSA·H_2O, toluene, reflux. (d) AD-mix α or β, $K_2OsO_2(OH)_4$, $CH_3SO_2NH_2$, *t*BuOH/H_2O (1/1, v/v), 0-20°C. (e) i) $CH_3C(OCH_3)_3$, PTSA·H_2O, CH_2Cl_2, 20°C; ii) TMSCl, CH_2Cl_2, 20°C; iii) K_2CO_3, MeOH, 20°C.

As we were unable to crystallise the two epoxides **22** to determine their absolute configuration, we employed the Mosher method on the corresponding diols **21**.[38] After selectively protecting the primary alcohol with *t*-butyldimethylsilyl chloride, each monoprotected diol **23** was converted into diastereomeric Mosher esters using either (*R*)-methoxy(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) or (*S*)-MTPA-Cl (**Scheme 3**). The four diastereomers (*R,X*)-**24** and (*S,X*)-**24**, where X represents the unassigned stereochemistry of the secondary alcohol, were obtained in 15 to 55% overall yield. The interpretation of the Δδ parameter in Mosher's model (**Figure S1**) clearly confirmed the presumed configurations, which was corroborated later by X-ray crystallography studies on crystals obtained from **21a** (**Figure S2**). As previously observed in the quinoline and pyridine series, the (*R*)- and (*S*)-enantiomers were derived from the use of AD-mix β and α respectively.[24,29]

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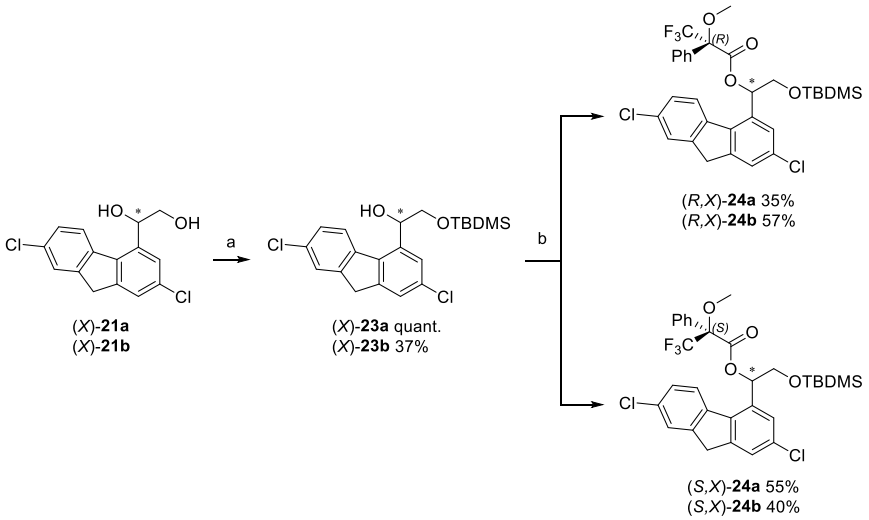
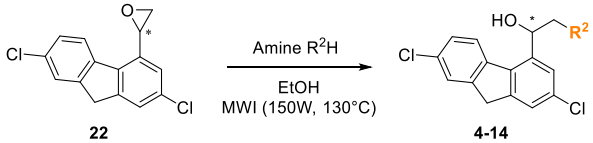
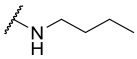


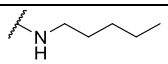
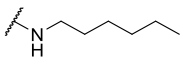
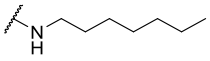
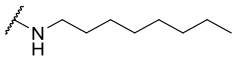
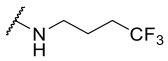
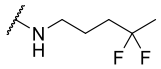
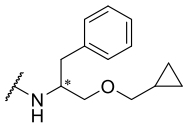
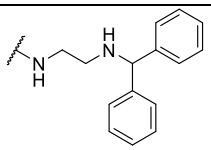
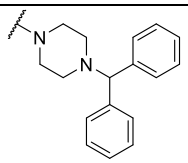
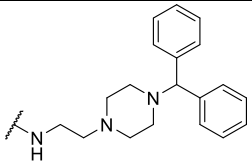
Scheme 3. Preparation of the Mosher's esters. Reagents and conditions: (a) TBDMSCl, DMAP, Et_3N, CH_2Cl_2, 20 °C. (b) (*R*) or (*S*)-MTPA, DMAP, EDCI, CH_2Cl_2, 0-20 °C.

After confirming the absolute configuration of the diols **21a** and **21b** and thus the epoxides **22a** and **22b**, regioselective nucleophilic substitution ring opening with various amines led to the aminoalcohol fluorene derivatives **4-14** (**Table 1**). The selection of amines for this study was based on previous results obtained on quinoline and 6-arylpyridine series.[24,29,32,33] Consequently, twenty-four aminoalcohol fluorenes were synthesised with overall yields ranging from 9% to 72%, and good enantiomeric (*ee*) or diastereoisomeric (*de*) excesses higher than 95%, except for four compounds (**Table 1, entries 13-15**, and **20**). X-ray crystals were obtained for (*S*)-**6**, confirming the absolute configuration previously established (**Figure S3**).

Table 1. Yields and enantiomeric or diastereoisomeric purities of aminoalcohol fluorenes.



Entry	Cpd	R^2	Yield (%)[a]	Overall yield (%)[b]	*ee* or *ed* (%)[c]
1	(*R*)-**4**		77	55	99
2	(*S*)-**4**		68	47	99
3	(*R*)-**5**		Quant.	72	99

4	(*S*)-**5**		83	57	99
5	(*R*)-**6**		Quant.	72	99
6	(*S*)-**6**		56	39	99
7	(*R*)-**7**		83	60	97
8	(*S*)-**7**		60	41	99
9	(*R*)-**8**		44	32	99
10	(*S*)-**8**		56	39	99
11	(*R*)-**9**		36	26	99
12	(*S*)-**9**		66	45	99
13	(*R*)-**10**[d]		14	10	88
14	(*S*)-**10**[d]		20	14	92
15	(*R,R*)-**11**		23	16	79
16	(*S,R*)-**11**		13	9	97
17	(*R,S*)-**11**		38	27	96
18	(*S,S*)-**11**		56	39	95
19	(*R*)-**12**		32	23	97
20	(*S*)-**12**		52	36	92
21	(*R*)-**13**		71	51	99
22	(*S*)-**13**		64	44	95
23	(*R*)-**14**		41	29	97
24	(*S*)-**14**		34	23	99

[a] Isolated yield in the final step.

[b] Calculated yield over six steps from **15**.

[c] Enantiomeric or diastereoisomeric excess. See **Supporting Information** for conditions.

[d] Hydrochloric salt.

Cpd: Compound.

In vitro antimalarial activity

All aminoalcohol fluorenes were evaluated for their *in vitro* antimalarial activity against *P. falciparum* 3D7 and W2 strains (**Table 2**). MQ, LM and chloroquine (CQ) were used as

references. LM was the most active with an IC_{50} value lower than 10 nM against the two strains. Aminoalcohol fluorenes **4-14** demonstrated good to excellent antimalarial activity, with IC_{50} values below 70 nM. Seventeen compounds displayed activity close to or below the reference LM against the two strains. Thus, the absence of the C9-substitution had no deleterious effect on the activity for most compounds on the strains used. The elongation of the amino aliphatic chain was well tolerated, with most compounds exhibiting IC_{50} values below 10 nM (**entries 1-15**). **Rac-5** and **Rac-8** showed comparable activity to their enantiopure aminoalcohol fluorenes. Thus, antimalarial activity can be either (i) identical between the two enantiomers and the racemate or (ii) the activity of the racemate corresponds to the average of the activity observed for the two enantiomers. **Rac-4** was 1.5 to 5 times more active than its two enantiomers (*R*)-**4** and (*S*)-**4**. This could be due to a potential synergistic effect between the two enantiomers. (*R*)-**6** was 5 to 7 times less active than its counterparts **rac-6** and (*S*)-**6** (**entries 7-9**), and **rac-7** was 2 to 20 times less effective than its enantiomers regardless of the strain possibly indicating an antagonistic effect between the enantiomers (**entries 10-12**).

The incorporation of fluorine atoms, a second stereogenic center or another secondary amine with a hydrophobic group (benzyl, benzhydryl) retained strong nanomolar activity (**entries 16-31**). However, the absence of secondary amines had a negative effect on activity, as highlighted by compound **13** containing the piperazine moiety. The two enantiomers of **13** were up to 3 and 39 times less potent than the enantiomeric pair **12** containing secondary amines against the 3D7 and the W2 strains respectively (entries **28-29** *versus* entries **26-27**). Contrary to our previous observations in the aminoalcohol quinoline series, there is no clear trend indicating that one enantiomer is superior to the other in terms of activity. For instance, (*S*)-**6** was 14 and 5 times more active than (*R*)-**6** against the 3D7 and W2 strains, respectively (**entries 8-9**). Conversely, (*R*)-**8** was more active than (*S*)-**8** by a factor of 6 on the 3D7 strain only (**entries 14-15**).

The synthesised aminoalcohol fluorenes often exhibited equal or even greater potency compared to the reference drugs (MQ, CQ and LM) and the quinoline leads (*S*)-**1**, (*S*)-**2** and (*S*)-**3**. This highlights the significant antimalarial potential of the fluorene core combined with the amino chains that we developed.

Table 2. *In vitro* antimalarial activity of aminoalcohol fluorenes **4-14**.

		IC_{50} ± SD (nM)[a]		**Eudysmic-ratio[b]**	
Entry	**Cpd**	***Pf*3D7[c]**	***Pf*W2[d]**	***Pf*3D7[c]**	***Pf*W2[d]**
1	**rac-4**	4.7 ± 0.8	11.5 ± 0.6		

2	(*R*)-**4**	23.6 ± 2.4	18.8 ± 1.2	1.0	0.64
3	(*S*)-**4**	23.3 ± 3.3	29.0 ± 2.6		
4	**rac-5**	3.6 ± 0.5	8.7 ± 0.2		
5	(*R*)-**5**	3.3 ± 0.4	3.7 ± 0.3	0.63	0.43 (*R*)
6	(*S*)-**5**	5.2 ± 1.2	8.6 ± 0.8		
7	**rac-6**	4.1 ± 0.1	6.0 ± 1.3		
8	(*R*)-**6**	31.1 ± 1.7	33.3 ± 0.9	0.07(*S*)	0.20 (*S*)
9	(*S*)-**6**	2.2 ± 0.2	6.5 ± 0.5		
10	**rac-7**	29.4 ± 6.9	18.6 ± 1.9		
11	(*R*)-**7**	0.9 ± 0.01	4.4 ± 0.4	0.60	0.94
12	(*S*)-**7**	1.5 ± 0.3	4.7 ± 0.2		
13	**rac-8**	2.1 ± 0.1	3.7 ± 0.6		
14	(*R*)-**8**	0.7 ± 0.1	5.1 ± 0.3	0.15 (*R*)	1.0
15	(*S*)-**8**	4.5 ± 0.2	5.0 ± 0.8		
16	(*R*)-**9**	4.4 ± 0.2	3.8 ± 0.6	0.53	0.92
17	(*S*)-**9**	8.3 ± 0.1	4.1 ± 0.1		
18	(*R*)-**10**[e]	8.5 ± 1.6	2.0 ± 0.3	0.60	0.30 (*S*)
19	(*S*)-**10**[e]	5.1 ± 0.9	0.6 ± 0.2		
22	(*R,R*)-**11**	3.8 ± 0.2	5.4 ± 0.7	0.30 (*R,R*)	0.52
23	(*S,S*)-**11**	11.2 ± 1.5	2.8 ± 0.01		
24	(*S,R*)-**11**	10.4 ± 1.4	5.4 ± 0.5	0.55	0.84
25	(*R,S*)-**11**	19.1 ± 9.9	6.4 ± 2.7		
26	(*R*)-**12**	10.9 ± 1.1	2.6 ± 0.6	0.73	0.69
27	(*S*)-**12**	8.0 ± 0.3	1.8 ± 0.2		
28	(*R*)-**13**	32.4 ± 10.1	57.9 ± 8.6	0.80	0.82
29	(*S*)-**13**	25.6 ± 7.9	70.2 ± 10.4		
30	(*R*)-**14**	14.2 ± 1.6	13.0 ± 1.8	0.73	1.0
31	(*S*)-**14**	19.5 ± 2.0	13.0 ± 0.7		
References					
36	MQ	22.4 ± 15.0	20.7 ± 1.3		
37	CQ	13.8 ± 5.6	163.6 ± 38.1		
38	LM	7.3 ± 5.4	4.1 ± 1.7		

[a] Results are mean ± standard deviation from three experiments. See **Supporting Information** for protocols.

[b] The eudysmic ratio is the IC_{50} ratio of the more active enantiomer on the less active enantiomer. The more active enantiomer is indicated in bracket if the ratio is < 0.5.
[c] *P. falciparum* strain with decreased sensibility to mefloquine and sensitive to chloroquine.
[d] *P. falciparum* strain sensitive to mefloquine and resistant to chloroquine.
[e] Hydrochloric salt.
ND: Not Determined.
Cpd: Compound.

In vitro cytotoxicity

The *in vitro* cytotoxicity of aminoalcohol fluorenes was evaluated against the HepG2 hepatocellular line, as recommended by the MMV, using mainly the Celltiter-Glo® method but also the MTT one (**Table 3, entries 15, 16, 27, 28, 30, 31**). Both methods yielded comparable results in our case and no significant difference was observed in the enantiomeric ratio of selectivity indexes (SIE) despite a 2-fold difference in the CC_{50} for compound **9** (**entries 15 and 16**).

Most aminoalcohol fluorenes exhibited cytotoxicity comparable to MQ and CQ, with CC_{50} values ranging from 2.7 to 27.1 µM. However, they were all more cytotoxic than LM. The introduction of fluorine atoms at the end of the alkyl chain or a second stereogenic center with a bulky group slightly reduced cytotoxicity, with CC_{50} values around 13 µM. Notably, replacing secondary amines with tertiary amines in **13** resulted in a substantial decrease in cytotoxicity by more than 17-fold compared to **12** (**entries 23-25** *versus* **entries 25-26**). Racemic and enantiomeric aminoalcohol fluorenes displayed similar cytotoxicity, except for (*R*)-**4**, which was 2 to 3-fold less cytotoxic than both the racemic and (*S*)-enantiomer (**entries 1-3**), and for (*R*)-**14**, which was 3-fold less cytotoxic than (*S*)-**14** (**entries 27-28**).

Since we were unable to distinguish the aminoalcohol fluorenes based only on cytotoxicity, we determined the selectivity index (SI) and the SIE for each *Pf* strain. To our delight, all the aminoalcohol fluorenes demonstrated excellent SI, ranging from 144 to 9714, due to strong nanomolar antimalarial activities. Furthermore, the SIE ratios often favoured the (*R*)-enantiomer, supporting our enantioselective synthesis strategy. Only the pairs **6** and **10** showed a ratio supporting the (*S*)-enantiomer. Compound **6** had the highest ratio among all compounds, with the (*S*)-enantiomer being 18 times and 6 times less cytotoxic than the (*R*)-enantiomer (**entries 7-8**).

Table 3. *In vitro* cytotoxicity and selectivity indexes of aminoalcohol fluorenes **4-14**.

		CC_{50} ± SD (μM)[a]	SI[b]		SIE ratio[c]	
Entry	**Cpd**	**HepG2**	***Pf*3D7**	***Pf*W2**	***Pf*3D7**	***Pf*W2**
1	**rac-4**	6.4 ± 0.8	1362	557		
2	(*R*)-**4**	20.9 ± 4.5	886	1112	2.4 (*R*)	3.7 (*R*)
3	(*S*)-**4**	8.7 ± 4.8	373	300		
4	**rac-5**	4.1 ± 1.2	1139	471		
5	(*R*)-**5**	6.3 ± 0.2	1909	1703	2.4 (*R*)	2.4 (*R*)
6	(*S*)-**5**	6.0 ± 2.0	800	698		
7	(*R*)-**6**	4.8 ± 1.0	154	144	18.6 (*S*)	6.7 (*S*)
8	(*S*)-**6**	6.3 ± 1.8	2864	969		
9	**rac-7**	5.7 ± 0.6	194	306		
10	(*R*)-**7**	6.7 ± 4.5	7444	1523	2.2 (*R*)	1.4
11	(*S*)-**7**	5.0 ± 1.6	3333	1064		
12	**rac-8**	5.9 ± 1.2	2809	1594		
13	(*R*)-**8**	6.8 ± 0.8	9714	1333	4.8 (*R*)	1.3
14	(*S*)-**8**	9.0 ± 1.7	2000	1800		
15	(*R*)-**9**	13.8 ± 5.5 (6.9 ± 0.4[d])	3136 (1568)	3631 (1815)	1.9 (1.8)	1.1 (1.1)
16	(*S*)-**9**	13.5 ± 8.0 (6.9 ±1.3[d])	1626 (831)	3293 (1682)		
17	(*R*)-**10**[f]	4.1 ± 0.1	482	2050	1.1	2.2 (*S*)
18	(*S*)-**10**[f]	2.7 ± 0.7	529	4500		
19	(*R,R*)-**11**	19.9 ± 7.9	5237	3685	4.2 (*R,R*)	1.3
20	(*S,S*)-**11**	13.8 ± 3.4	1232	4929		
21	(*S,R*)-**11**	13.4 ± 2.5	1288	2481	1.8	1.1
22	(*R,S*)-**11**	13.8 ± 2.2	723	2156		
23	(*R*)-**12**	3.2 ± 0.3	294	1231	1.3	1.3
24	(*S*)-**12**	3.0 ± 0.1	375	1667		
25	(*R*)-**13**	>50	>1543	>863	ND	ND
26	(*S*)-**13**	>50	>1950	>712		
27	(*R*)-**14**	27.1 ± 5.8[d]	1908	2084	4.6 (*R*)	3.4 (*R*)
28	(*S*)-**14**	8.0 ± 0.8[d]	410	615		
References						
29	MQ	3.3 ± 0.7	147	159		
30	CQ	24.1 ± 0.8[d]	1746	147		

31	LM	> 100[d]	>13000	>24000		

[a] 50% cytotoxic concentration in micromolar using the CelltiterGlo® method. Results are mean ± standard deviation from three independent experiments. See **Supporting Information** for protocols.

[b] Selectivity indexes (SI) are the ratio of CC_{50} on IC_{50}.

[c] SIE ratio is SI of the more selective enantiomer on the less selective enantiomer. The more selective enantiomer is indicated in bracket if ratio is > 2.

[d] 50% cytotoxic concentration in micromolar using the MTT method. Results are mean ± standard deviation from three independent experiments. See **Supporting Information** for protocols.

[e] Results are mean ± standard deviation from two independent experiments.

[f] Hydrochloric salt.

ND: Not Determined.

Cpd: Compound.

Drug-likeness prediction

The Lipinski's rule of five (Ro5) is used to predict the drug-likeness of orally administered compounds.[39] Ro5 includes standard ranges for the molecular weight (MW < 500), the number of hydrogen bond donor (HBD < 5), the number of hydrogen bond acceptor (HBA < 10), and the predicted octanol/water partition coefficient (clogP < 5). According to the results presented in **Table 4**, most synthesised aminoalcohol fluorenes satisfied drug-likeness conditions with zero (**10**) or one violation (**4-8**, and **11**). Compounds **4-8** and **11** exhibited high clogP values, ranging from 5.1 to 6.8, which were lower than that of LM (clogP = 8.1). Compounds **12**-**14** containing an EPI scaffold displayed two violations of the Ro5 due to clogP and MW exceeding the standard limits.

Compounds with high clogP value are not incompatible with oral administration for malarial treatment since LM is marketed and used in ACT for instance. Indeed, to predict a drug's behaviour in a biological system, clogD is often considered as a more relevant descriptor of lipophilicity than clogP since it accounts for the ionisation state of the compound. At blood serum pH, all compounds are monoprotonated, which attenuates their high lipophilicity. Notably, all compounds displayed lower $clogD_{7.4}$ values than LM, ranging from 1.8 to 4.2, except for **11** and **13**.

Independent of MW, reduced molecular flexibility measured by the number of rotatable bonds (ROTB), and low polar surface area (PSA) are also important predictors of good oral bioavailability as promoted by Veber et al.[40] Reduced PSA (< 140 Å²) correlates with an increased permeation rate, while higher ROTB count negatively impacts permeation. A

threshold of 10 ROTB is considered a prerequisite for oral bioavailability. Interestingly, all the aminoalcohol fluorenes adhered to Veber's rules as well as the reference drugs. This suggests that the compounds could have good permeability across cell membranes despite the high lipophilicity identified by Ro5.

Table 4. Physicochemical descriptors of aminoalcohol fluorenes **1-14**.

	Ro5						Veber's rule	
Cpd	**MW (g/mol)**	**HBA[a]**	**HBD[a]**	**cLogP[b]**	**pKa**	**cLogD$_{7.4}$[b]**	**PSA (Å^2)[a]**	**ROTB[a]**
Previous compounds								
1	394.3	9	2	3.8	9.4	1.8	45.1	9
2	472.4	10	2	3.5	7.5	3.1	54.4	10
3	533.5	10	2	4.2	8.6, 9.3	1.0	57.2	11
New compounds								
4	350.3	2	2	5.4	9.3	3.5	32.3	6
5	364.3	2	2	5.8	9.9	3.5	32.3	7
6	378.3	2	2	6.2	9.9	3.5	32.3	8
7	392.4	2	2	6.5	9.8	3.5	32.3	9
8	406.4	2	2	6.8	9.9	3.5	32.3	10
9	404.2	5	2	5.1	8.7	3.8	32.3	7
10	400.3	4	2	4.1	9.9	1.6	32.3	7
11	482.4	3	2	6.7	8.1	5.9	41.5	10
12	503.5	3	3	6.0	9.1, 9.3	2.4	44.3	9
13	529.5	3	1	6.3	1.9, 7.5	6.0	26.7	6
14	572.6	4	2	5.8	2.1, 6.2, 9.0	4.2	38.7	9
References								
CQ	319.9	2	1	4.3	8.1, 11.1	0.1	28.2	8
MQ	378.3	9	2	3.5	1.4, 8.7	2.1	45.1	4
LM	528.9	2	1	8.1	9.0	6.5	23.5	10

[a] Calculated by the open source software SwissADME.

[b] Calculated by the software Pallas 3.5.

Cpd: Compound.

To understand the pharmacokinetic (ADMET parameters) of the aminoalcohol fluorenes, we selected various key indicators using the QikProp module of the Schrodinger Suite. These include: i) QPlogS, which reflects aqueous solubility and therefore absorption; ii) QPPCaco2, which indicates permeability (absorption, distribution); iii) the number of metabolic spots for metabolism; iv) QPlogBB, which indicates accessibility for the central nervous system; and v) QPlogHERG, which reflects potential cardiac toxicity. The indicator values for compounds **1-14** along with the standard limits are reported in **Table 5**. Jorgensen's rule of three (ro3) was used to predict the oral bioavailability likelihood, including the aqueous solubility (QPlogS > -5.7 mol.L^{-1}), the gut/blood barrier permeability (QPPCaco2 > 22 nm.s^{-1}), and the number of primary metabolites (< 7) parameters.[41] Interestingly, the four compounds **4-6** and **10** showed good oral absorption meeting all ro3 criteria. Due to high lipophilicity, aqueous solubility (QPlogS) levels were low for compounds **7-9** and **11-13**, as well as for LM. Compounds **12**-**14** containing an EPI scaffold were also out of the recommended range for metabolic reactions.

All aminoalcohol fluorenes showed QPlogBB within the recommended range, suggesting good permeability across the blood/brain barrier. This was reinforced by PSA values below 80 Å^2. Similar to MQ, the aminoalcohol fluorenes could potentially exert their antimalarial activity in the brain, in cases of cerebral malaria, or show neurological side effects in the worst scenario.

Cardiotoxicity is a possible side effect of many drugs, causing heart problems as well as sudden cardiac death. It is therefore important to assess this parameter early in the lead discovery to quickly consider chemical scaffold modifications.[42] One of the mechanisms involved is blockage of voltage-gated potassium channels called hERG channels. QPlogHERG is a common descriptor used to predict the IC_{50} value for the blockage of these channels. All synthesised aminoalcohol fluorenes and previous lead compounds showed values below -5, which raises concerns. Compound **10** had the lowest predicted toxicity, with QPlogHERG = -5.68. In contrast, compounds with an EPI scaffold in quinoline (**3**) and fluorene (**12-14**) series were predicted to be the most cardiotoxic, as they mimic the pharmacophore involved in channel blockage.[42] Interestingly, the three marketed drugs also displayed QPlogHERG values below -5. It is important to note that these values are predicted and should be used to guide the medicinal chemist. *In vitro* measurements are essential to accurately assess cardiotoxicity.

Table 5. Key indicators for pharmacokinetic parameters of aminoalcohol fluorenes **1-14**.

	Jorgensen's ro3				
Cpd	**QPlogS (mol.L^{-1})[a]**	**QPPCaco (nm.s^{-1})[a]**	**Primary metabolites[a,b]**	**QPlogBB[a]**	**QPlogHERG (mol.L^{-1})[a]**

Standard limits	-6.5 – 0.5	< 25 (poor) > 500 (great)	< 7	-3 – 1.2	≤ -5
Previous compounds					
1	-5.21	861	2	0.51	-5.99
2	-5.58	1140	4	0.61	-6.73
3	-6.39	169	6	0.61	-9.25
New compounds					
4	-4.84	913	4	0.42	-6.11
5	-5.00	927	4	0.37	-6.06
6	-5.27	950	4	0.32	-6.06
7	-5.92	1100	4	0.30	-6.55
8	-6.36	1004	4	0.18	-6.72
9	-5.76	911	4	0.71	-6.23
10	-4.48	952	4	0.61	-5.68
11	-6.67	1065	5	0.28	-7.54
12	-6.17	213	8	0.54	-9.33
13	-6.73	303	8	0.92	-9.07
14	-5.01	55	9	0.59	-9.55
References					
CQ	-4.03	1506	4	0.43	-5.81
MQ	-4.72	975	1	0.93	-5.50
LM	-8.31	1548	3	0.51	-7.25

[a] Calculated with QikProp.

[b] List of reactions available in **Supporting Information**.

Cpd: Compound.

Conclusion

This work has led to a better understanding of aminoalcohol fluorenes in order to broaden their potential in antimalarial treatments. We developed a racemic and an enantiopure synthesis to obtain twenty-eight simplified analogues of LM containing various aminochains previously identified in quinoline and 6-arylpyridine series. All compounds were evaluated for their antimalarial activity against the two strains *Pf*3D7 and *Pf*W2. The results showed that all compounds displayed excellent *in vitro* nanomolar activities (IC_{50} < 70 nM) against both strains, with most showing greater activity than the reference drugs MQ, CQ, and LM. SAR analysis indicated that all studied aminochains were well tolerated, confirming their importance in the

antimalarial potency of arylaminoalcohols. Interestingly, C9-substitution was not essential for antimalarial activity. Unlike the quinoline and 6-arylpyridine series, no clear enantiomeric selectivity was observed. Toxicity assessment on the HepG2 cell line showed moderate cytotoxicity, but excellent SI due to the strong antimalarial potency. Moreover, the LM analogues **4-14** demonstrated acceptable predicted drug-likeness behaviour and predicted oral bioavailability despite high lipophilicity. Among all aminoalcohol fluorenes, (*S*)-**10** is the most promising with a strong antimalarial activity (IC_{50} < 5 nM on both strains), an excellent SI (> 500), an eudysmic ratio favouring the (*S*)-enantiomer over the (*R*)-enantiomer, and a very good drug-likeness prediction score. Notably, (*S*)-**10** meets key thresholds established by the MMV for leads in antimalarial drug discovery, including IC_{50} below 100 nM, SI higher than 100, and favourable physicochemical properties.[43] Further studies are underway to confirm the *in vivo* antimalarial activity in mice, the pharmacokinetic properties and to explore the mode of action of this promising compound.

Authors contribution

CT wrote the manuscript. CT, JS, RMu, RMo and AH performed the synthesis, purification, and characterisation of all compounds disclosed in this manuscript. CT, RMu, CD, PA, AT designed and performed the biological studies described in this manuscript. MM and JG designed and performed X-ray crystallography studies. ADK and PS supervised the scientific studies and edited the manuscript.

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Conflicts of interest

The authors declare no conflicts of interest.

Supporting Information

Supplementary data related to this article (chemistry, Mosher's analysis, *in vitro* antimalarial activity and cytotoxicity, ^{1}H NMR, ^{13}C NMR, HRMS spectra and purity analyses by HPLC of compounds **4**-**14**) are available online. The authors have cited additional references (44-49) within the Supporting Information.

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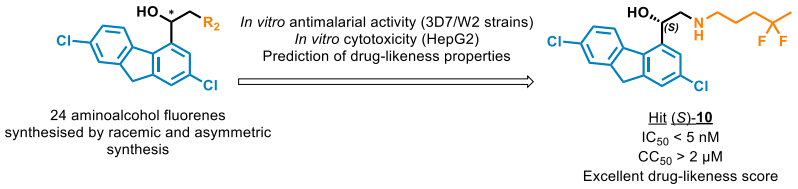
Table of contents

Considering the importance of developing new antimalarial drugs, we provide new insights in enantiopure aminoalcohol fluorenes as simplified analogs of lumefantrine. All compounds possess potent *in vitro* antimalarial activity against two strains of *P. falciparum* and good selectivity indexes. Structure-activity relationship studies identified one hit **10** with promising drug-likeness properties.



Antimalarial; aminoalcohol fluorenes; asymmetric synthesis; lumefantrine; medicinal chemistry.