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Synthesis and comparison of antiplasmodial activity of (+), (−) and racemic 7-chloro-4-(*N*-lupinyl)aminoquinoline

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ABSTRACT

Recently the *N*-(−)-lupinyl-derivative of 7-chloro-4-aminoquinoline ((−)-AM-1; 7-chloro-4-{*N*-[(1*S*,9a*R*)(octahydro-2*H*-quinolizin-1-yl)methyl]amino}quinoline) showed potent in vitro and in vivo activity against both Chloroquine susceptible and resistant strains of *Plasmodium falciparum*. However, (−)-AM-1 is synthesized starting from (−)-lupinine, an expensive alkaloid isolated from *Lupinus luteus* whose worldwide production is not sufficient, at present, for large market purposes. To overcome this issue, the corresponding racemic compound, derived from synthetic (±)-lupinine was considered a cheaper alternative for the development of a novel antimalarial agent. Therefore, the racemic and the 7-chloro-4-(*N*-(+)-lupinyl)aminoquinoline ((±)-AM-1; (+)-AM-1) were synthesized and their in vitro antimalarial activity and cytotoxicity compared with those of (−)-AM-1. The (+)-lupinine required for the synthesis of (+)-AM-1 was obtained through a not previously described lipase catalyzed kinetic resolution of (±)-lupinine. In terms of antimalarial activity, (±)-AM1 and (+)-AM1 demonstrated very good activity in vitro against both CQ-R and CQ-S strains of *P. falciparum* (range IC_{50} 16–35 nM), and low toxicity against human normal cell lines (therapeutic index >1000), comparable with that of (−)-AM1. These results confirm that the racemate (±)-AM1 could be considered as a potential antimalarial agent, ensuring a decrease of costs of synthesis compared to (−)-AM1.

1. Introduction

Malaria is an infectious disease causing more than 500 million cases and approximately 700 thousands deaths every year. The majority of these cases are due to *Plasmodium falciparum* and occur mainly in sub-Saharan Africa. *Plasmodium vivax,* the second most frequent human malaria species causes more than 80 million clinical episodes particularly in Asia, where it accounts for at least half of the malaria burden.[1]

Chloroquine (CQ), one of the most efficacious, safe and cheap antimalarial drugs, has been effectively used for decades against both *Plasmodium* species. Unfortunately now it is largely ineffective against *P. falciparum* because of widespread parasite resistance.[2] New drugs for combating parasite resistant strains are needed, even more now that the efforts are directed toward elimination and eradication of malaria by 2050.[3] Quinoline-type antimalarials remain an attractive class of compounds because their mechanism of action and resistance are unrelated and resistance has emerged very slowly over time. Recently, several new 4-aminoquinoline derivatives have been synthesized and studied. Among them, AQ-13 and ferroquine are under development as novel antimalarial drugs and entered into Phase 2 clinical trials.[4–6]

In the last few years, the research of our group has been directed to the study of new quinolizidinyl and quinolizidinyl-alkyl derivatives of 7-chloro-4-aminoquinoline.[7,8] Compounds highly effective in vitro against both the D-10 (CQ-sensitive, CQ-S) and W-2 (CQ-resistant, CQ-R) strains of *P. falciparum*[7] and in vivo in the murine *P. berghei* model,[9] have been obtained. One of these compounds, (−)-AM1 has been selected for further characterization: (−)-AM1 is a semisynthetic product that can be obtained in 4 steps with good yield (52.5%) as a pure enantiomer, starting from (−)-lupinine, which is transformed into (−)-aminolupinane ((1*S*, 9a*R*)-octahydro-2*H*-quinolizine-1-methanamine) and coupled with 4,7-dichloroquinoline to give the final compound, as previously reported by Boido Canu et al.[10] (Scheme 1).

Lupinine is an alkaloid present in several *Leguminosae* (*Lupinus luteus, L. hispanicus*) and in *Chenopadiaceae* (*Anabasis aphylla*). It is the major alkaloid of bitter *Lupinus luteus* that was cultivated in central Europe for fixing nitrogen in soil. Unfortunately, the extension of this cultivation is presently very limited because 'sweet'

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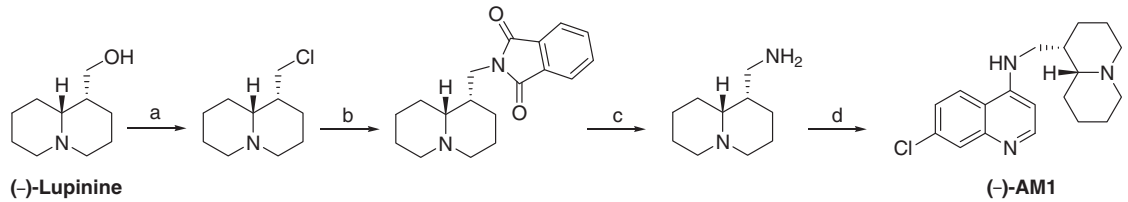
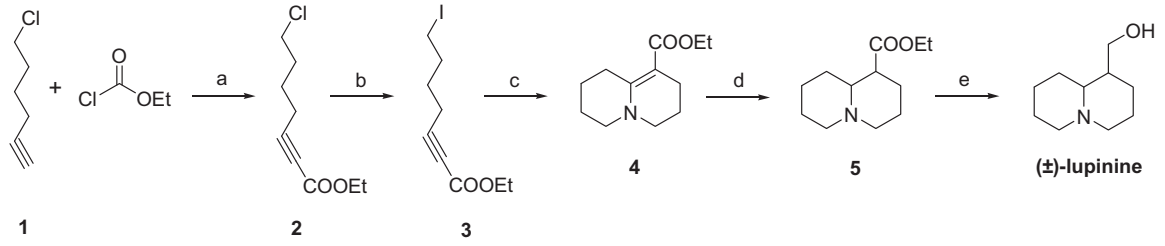


Scheme 1. Reagents and conditions: (a) $SOCl_2$, $CHCl_3$, reflux; (b) potassium phthalimide, DMSO, N_2, 145 °C; (c) 6 N HCl, reflux; (d) 4,7-dichloroquinoline, phenol, 180 °C.



Scheme 2. Reagents and conditions: (a) *n*-BuLi, Et_2O, −30 °C; (b) NaI, CH_3COCH_3, reflux; (c) 3-chloropropylamine.HCl, MeCN, K_2CO_3, 4 Å MS, reflux; (d) $NaBH_4$, CH_3OH, 0–5 °C; (e) $LiAlH_4$, Et_2O, rt.

varieties of *Lupinus* (*L. albus, L. luteus*), without alkaloids, are now preferred for human consumption or for animal feeding. Lupinine from *Anabasis* is available commercially, however it is quite expensive and not sufficient for drug production. Therefore, although the synthesis of (−)-AM1 from lupinine is quite straightforward, the limited availability and the cost of the starting material represent unsuited features for a potential antimalarial agent.

Since the activity of 4-aminoquinoline antimalarials does not seem to be correlated with the chirality of the drug, the presence of a specific absolute configuration on the basic head should not be necessary. Therefore, we synthesized and investigated the activity of the racemate of AM1 ((±)-AM1), thus ensuring a production independent from the natural source and, consequently, cheaper. However, since a racemic mixture can be used in therapy only if both enantiomers display comparable biological and toxicological profiles, the synthesis and the pharmacological characterization of racemic AM1 and (+)-AM1 were also planned in comparison with (−)-AM1.

In this paper, we report the synthesis and the in vitro antiplasmodial activity against different strains of *P. falciparum* of the racemate of AM1 and the enantiomers. The cytotoxicity against human cells is also reported.

2. Chemistry

According to the synthetic pathway followed for (−)-AM1, the synthesis of (±)-AM1 and (+)-AM1 required as starting material (±)-lupinine and (+)-lupinine, respectively, which are not commercially available.

In order to obtain pure (+)-lupinine, two different synthetic strategies could be adopted: the stereoselective synthesis of (+)-lupinine or the total synthesis of racemic lupinine, followed by the resolution of the two enantiomers.

It has been reported that (+)-lupinine can be obtained in seven steps starting from (−)-pipecolic acid.[11] Following this procedure (+)-lupinine was obtained with an enantiomeric excess of 50–60%, which increased up to 80% by repeated fractional crystallizations; thus, this synthetic route resulted low yielding and not economically advantageous and it was, therefore, abandoned.

The total synthesis of racemic lupinine, followed by the resolution of the two enantiomers was then attempted.

Key intermediate in the synthesis of (±)-lupinine is the unsaturated quinolizidine ester **4** (Scheme 2). This compound has been obtained by reaction of the alkyliodide **3** with 3-chloropropylamine in MeCN assisted with K_2CO_3, which undergoes a sequential SN2/Michael addition/SN2/SN2 reaction process.[12]

Selective reduction of the double bond by $NaBH_4$,[13] followed by $LiAlH_4$ reduction of the ester afforded rac-lupinine with an overall yield of 30%.

(±)-AM1 was obtained from (±)-lupinine by applying the four steps synthetic scheme shown in Scheme 1 for the synthesis of (−)-AM1.

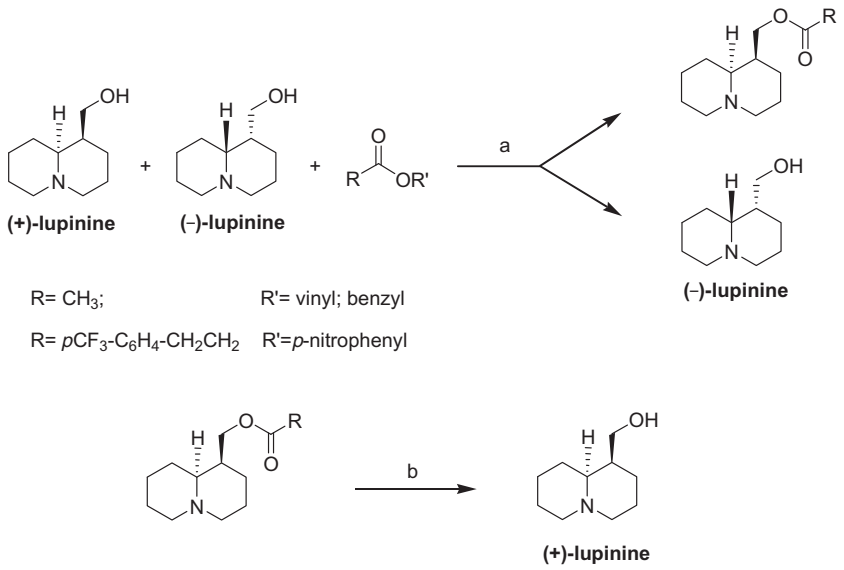
To obtain (+)-AM1 by resolution, two different routes were possible: either the resolution of (±)-AM1 or the resolution of (±)-lupinine, followed by conversion of (+)-lupinine into (+)-AM1. We decided to attempt the second alternative considering that: (a) the presence of an alcoholic functional group allows the use of several chiral auxiliaries; (b) in a synthetic process, it is always preferable to have the lowest yield step as early as possible.

Although the resolution of racemic primary alcohols is well known, resolution of lupinine could be a difficult task, mainly due to the lack of aromatic rings. An interesting approach is the kinetic resolution of racemic alcohols, through lipase-catalyzed asymmetric transesterification in organic solvents. However, this method is more efficient when applied to secondary alcohols, while kinetic resolution of racemates of primary alcohols is more difficult to achieve, due to the lower enantioselectivity of lipases towards chiral primary alcohols.[14–16]

In lipase catalyzed reactions, the type of acyl donor plays an important role in the reaction kinetics (or in the selectivity reaction).[17–20] We have studied three different acyl donors, 4-nitrophenyl 3-[4-(trifluoromethyl)phenyl]propanoate,[17] benzyl acetate and vinyl acetate,[21] using toluene as solvent. During the transesterification reaction, the lipases (PS or AK) are initially acylated by the acyl donor, and the acylated lipase then reacts enantioselectively with an alcohol (Scheme 3).

As shown in Table 1, first we used 4-nitrophenyl 3-[4-(trifluoromethyl)phenyl]propanoate as acyl donor, but we obtained (+)-lupinine with only 71% of ee[22]

Using vinyl acetate as acyl donor, we performed enzymatic resolution yielding different ee depending by different incubation/reaction times.



Scheme 3. Reagents and conditions: (a) Amano Lipase, toluene; (b) 1 N NaOH in MeOH.

Table 1
Enzymatic resolution conditions[a]

Entry	Lipase	Acyl donor	Incubation time	Reaction time	Lupinine $[\alpha]_D^{20}$	ee%[b]
1	PS	4-Nitrophenyl 3-[4-(trifluoromethyl)phenyl] propanoate	24 h	24 h	(+)14,9	71%
2	PS	Vinyl acetate	24 h	120 h	(+)17,7	82%
3	AK	Vinyl acetate	24 h	24 h	(+)13,3	62%
4	AK	Vinyl acetate	19 h	15 h	(+)18,6	87%
5	AK	Vinyl acetate	19 h	10 h	(+)18,6	87%
6	**AK**	**Vinyl acetate**	**19 h**	**5 h**	**(+)19.1**	**89%**
7	PS	Benzyl acetate	24 h	120 h	—	—
8	AK	Benzyl acetate	24 h	120 h	—	—

[a] For details see the Experimental Section.
[a] Ee was calculated assuming the value (+)21.5 as 100%. A pure sample of natural (−)lupinine has $[\alpha]_D^{20}$ −21.5 (*C* = 1, EtOH).[22]

Table 2
In vitro antimalarial and cytotoxic activities of AM1 racemate and its enantiomers

Compound	*P. falciparum* IC_{50} ± SD[a] (nM)				Cytotoxicity IC_{50}[b] (nM)	
	D-10	3D7	W-2	Pf-Kenya[c]	HMEC[d]	HDF[e]
(±)-AM1	16.15 ± 5.18	18.55 ± 7.70	35.44 ± 8.34	19.43 ± 8.64	>60,000	24,584
(−)-AM1	23.98 ± 8.83	28.14 ± 17.00	53.67 ± 22.72	29.20 ± 3.77	>49,000	22,616
(+)-AM1	17.49 ± 8.85	21.91 ± 0.29	35.77 ± 16.94	18.92 ± 8.82	>60,000	26,918
CQ	23.72 ± 13.99	16.92 ± 2.11	437.62 ± 93.47	241.30 ± 41.13	>38,000	>38,000

[a] pLDH method, mean of three different experiments.
[b] MTT method.
[c] *P.falciparum* strain isolated in Milan from a patient coming from Kenya, adapted to in vitro growth.
[d] Human endothelium.
[e] Human fibroblasts.

Esterification did not occur with benzylacetate as acyl donor, in presence of both lipases (PS and AK).

The best results (89% ee) were obtained after 19 h of incubation of Lipase AK with vinyl acetate followed by 5 h of reaction with (±)-lupinine.

To be noted that higher enantiomeric eccess (98.6%; $[\alpha]_D^{20}$ +21.17) was obtained by submitting a second time the enriched (+)-lupinine ($[\alpha]_D^{20}$ +13,4) to the resolution with lipase AK and vinyl acetate (19 h of incubation and 7 h of reaction).

Finally (+)-AM1 was obtained starting from (+)-lupinine, according to the same procedure used for (−)-AM1.[10]

3. Results and discussion

Racemic AM1, (−)-AM1 and (+)-AM1 (ee >80%) were tested in vitro against two CQ-R (W-2 and the Pf Kenia isolate) and two CQ-S (D10–3D7) strains of *P. falciparum*. Their antimalarial activity was quantified as inhibition of parasite growth[23] and was compared to the cytotoxicity[24] on mammalian cells of human origin (Table 2).

As shown, the antiplasmodial activities of (±)-AM1 and of the two enantiomers against the four strains, expressed as $IC_{50,}$ were in the range of 16–53 nM and were not significantly different each

other. They were equally active against CQ-R (range 19–53 nM) and CQ-S (range 16–28 nM) strains of *P. falciparum*, with no evidence of cross-resistance with CQ.

Racemic AM1 and the two enantiomers demonstrated low toxicity against human normal cells with a selectivity index generally >1000.

These results show an equivalent in vitro behaviour of the two enantiomers of AM1 and make reasonable the development of the cheaper racemate as a potential antimalarial drug. In vivo studies are required to confirm these first results.

4. Conclusions

We have identified the best conditions to synthesize (±)-AM1 and (+)-AM1 starting, respectively, from (±)-lupinine and (+)-lupinine, which was obtained through the enzymatic resolution of the former. The racemic AM1 and the (+)-enantiomer of AM1 display the same activity against *P. falciparum* and lack of toxicity as described previously for the (−)-enantiomer. These data indicate that racemic AM1 could be considered as a potential new antimalarial lead with decrease costs of synthesis compared to (−)-AM1 and broad spectrum of activity against different *Plasmodium* strains.

5. Experimental

5.1. General

Amano Lipases PS (*Pseudomonas cepacia, ⩾30,000 U/g*) and AK (*Pseudomonas fluorescens, ⩾20,000 U/g)* were supplied by Aldrich and were employed without any previous treatment. All commercially available solvents and reagents were used without further purification, unless otherwise stated. Purification of products and separations of esters and alcohols after enzymatic conversions were performed on silica gel 60 Merck. Melting points were determined with a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Varian Mercury 300VX spectrometer, using $CDCl_3$ as solvent; high-resolution mass spectra (HRMS) on a APEX II ICR-FTMS Bruker Daltonics mass spectrometer in positive electro spray ionization (ESI). Optical rotations were measured on a polarimeter Jasco P-1010 in EtOH at 20 °C.

5.2. (1*RS*, 9a*RS*)-Octahydro-2*H*-quinolizine-1-methanol; (±)-Lupinine

Sodium borohydride (2.23 g; 59.13 mmol) was added in small portions, during 2 h, to a solution of 3,4,6,7,8,9-hexahydro-2*H*-quinolizine-1-carboxylic acid ethyl ester, prepared following the method described by Cai et al.[12] (2.6 g; 12.42 mmol) in anh. MeOH (70 ml), kept between 0 and 5 °C and stirred under N_2. The reaction mixture was then diluted with water (300 ml) and extracted with ether (twice). The combined organic extracts were dried over anh. Na_2SO_4 and evaporated to dryness. A slight yellow oil was obtained (2.3 g; 10.89 mmol; 88.5%), which was dissolved in anh. ether (60 ml) and added dropwise to a suspension of $LiAlH_4$ (3.5 g; 94.52 mmol). The mixture was stirred at rt, under N_2, for 1.5 h. After cooling in an ice bath, wet ether was added dropwise to the mixture, followed by water. After filtration, the two layers were separated and the aqueous phase was saturated with Na_2CO_3 and extracted with ether. The combined ether extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. A slight yellow oil was obtained which crystallizes after treatment with hexane. Yield 81%; mp 56.5–57.8 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.25 (br s, 1H), 4.14 (dd, J = 4.13, 10.45 Hz, 1H), 3.68 (d, J = 10.72 Hz, 1H), 2.85–2.75 (m, 2H), 2.25–1.95 (m, 3H), 1.90–1.67 (m, 4H), 1.61–1.42 (m, 6H), 1.35–1.18 (m, 1H).

5.3. Enzymatic resolution: general method

Step 1: *(+)-Lupinyl ester formation.* The lipase (10 mg/mmol of lupinine) was added to a solution of racemic lupinine (1.77–5.9 mmol) in toluene (1.8 ml/mmol of lupinine) and the mixture was stirred for 19–24 h in the open flask put into a desiccators over anh. $CaCl_2$. Acyl donor (3.7 equiv) was added to the reaction flask, which was immediately sealed with a septum. The mixture was stirred for 5–120 h at rt. The reaction was quenched by filtration and the filtrate was concentrated under reduced pressure. The residue was chromatographed on a silica gel column using ethyl acetate–methanol–NH_3 in gradient as eluent to give the (+)-lupinine ester as white solid.

Step 2: Ester hydrolysis. The ester (1 equiv) was dissolved in 1 N NaOH in MeOH (3 equiv) at 0 °C and the reaction mixture was stirred at room temperature for 1 h. When the reaction was completed, the solution was concentrated; water was added and the aqueous phase was extracted with ethyl acetate. The combined organic extracts were dried over anhydrous Na_2SO_4, evaporated to dryness and the residue was washed with hexane to give (+)-lupinine as a white solid.

Experimental conditions and results of enzymatic resolution are collected in Table 1.

5.3.1. Experiment 1

Amano PS (282 mg) was added to a solution of racemic lupinine (560 mg, 3.32 mmol) in 34 ml of toluene in the reaction flask containing a magnetic stirring bar. The mixture in the open flask was stirred for 24 h in a sealed container over saturated $CaCl_2$. 4-Nitrophenyl 3-[4-(trifluoromethyl)phenyl]propanoate (1.127 g, 3.32 mmol) was added to the reaction flask, which was immediately sealed with a septum. The mixture was stirred for 24 h at rt. The reaction was quenched by filtration and the filtrate was concentrated under reduced pressure. The residue was chromatographed on a silica gel column using ethyl acetate–methanol–-NH_3 in gradient as the eluent, to give 128 mg of ester as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.57 (d, J = 8.05 Hz, 2H), 7.34 (d, J = 7.91 Hz, 1H), 4.47–4.16 (m, 2H), 3.04 (t, J = 7.52 Hz, 2H), 2.97–2.81 (m, 2H), 2.68 (t, J = 7.59 Hz, 2H), 2.21–1.15 (m, 15H).

The produced ester was hydrolyzed (1 N NaOH in MeOH, 3.5 ml) to give (+)-lupinine (73.3 mg, 0.18 mmol, $[\alpha]_D^{20}$ +14.9 (c = 1 in EtOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.25 (br s, 1H), 4.17–4.11 (m, 1H), 3.68 (d, J = 10.45 Hz, 1H), 2.85–2.77 (m, 2H), 2.19–1.97 (m, 3H), 1.95–1.67 (m, 4H), 1.63–1.45 (m, 6H), 1.31–1.20 (m, 1H).

5.3.2. Experiment 2

Amano PS (60 mg) was added to a solution of racemic lupinine (1 g, 5.9 mmol) in 10.6 ml of toluene and the mixture was stirred for 24 h. Vinyl acetate (2 ml, 21.7 mmol) was added and the mixture was stirred for 120 h at rt. After purification, were obtained 300 mg of ester (1.41 mmol) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.38–4.33 (m, 1H), 4.22–4.16 (m, 1H), 2.84–2.80 (d, J = 10.8 Hz, 2H), 2.24–1.73 (m, 10H), 1.69–1.30 (m, 6H), 1.30–1.24 (m, 1H).

The ester was hydrolyzed, according to general procedure (step 2), with 5 ml of 1 N NaOH in MeOH, to give (+)-lupinine (160 mg, 66%). $[\alpha]_D^{20}$= +17.67 (c = 1 in EtOH).

5.3.3. Experiment 3

Amano AK (30 mg) was added to a solution of racemic lupinine (500 mg, 2.95 mmol) in 5 ml of toluene and the mixture was stirred for 24 h. Vinyl acetate (1 ml, 10.9 mmol) was added and the mixture was stirred for 24 h at rt. After purification, were obtained 360 mg of ester (1,41 mmol) as a white solid.

The ester was hydrolyzed, with 6 ml of 1 N NaOH in MeOH, to give (+)-lupinine (150 mg, 63%). $[\alpha]_D^{20}$ +13.4 (c = 1 in EtOH).

5.3.4. Experiment 4

Amano AK (18 mg) was added to a solution of racemic lupinine (300 mg, 1.77 mmol) in 3 ml of toluene and the mixture was stirred for 19 h. Vinyl acetate (0.6 ml, 6.5 mmol) was added and the mixture was stirred for 5 h at rt. After purification, were obtained 104 mg of ester (0.49 mmol) as a white solid.

The ester was hydrolyzed with 1.6 ml of 1 N NaOH in MeOH, to give (+)-lupinine (50 mg, 60%); $[\alpha]_D^{20}$ +19.1 (c = 1 in EtOH).

5.3.5. Experiment 5

Amano AK (20 mg) was added to a solution of racemic lupinine (340 mg, 2 mmol) in 3 ml of toluene and the mixture was stirred for 19 h. Vinyl acetate (0.68 ml, 7.4 mmol) was added and the mixture was stirred for 15 h at rt. After purification, were obtained 224 mg of ester (1.06 mmol) as a white solid.

The ester was hydrolyzed with 5.3 ml of 1 N NaOH in MeOH, to give (+)-lupinine (110 mg, 61%). $[\alpha]_D^{20}$ +18.6 (c = 1 in EtOH).

5.3.6. Experiment 6

Amano AK (18 mg) was added to a solution of racemic lupinine (300 mg, 2 mmol) in 3 ml of toluene and the mixture was stirred for 19 h. Vinyl acetate (0.6 ml, 6.5 mmol) was added and the mixture was stirred for 10 h at rt. After purification, were obtained 183 mg of ester (0.87 mmol) as a white solid.

The ester was hydrolyzed with 4.3 ml of 1 N NaOH in MeOH, to give (+)-lupinine (95 mg, 60%). $[\alpha]_D^{20}$ +18.6 (c = 1 in EtOH).

5.3.7. Experiment 7

Amano PS (30 mg) was added to a solution of racemic lupinine (500 mg, 2.95 mmol) in 5 ml of toluene and the mixture was stirred for 24 h. Benzyl acetate (1.6 ml, 10.9 mmol) was added and the mixture was stirred for 96 h at rt but the transesterification did not occur.

5.3.8. Experiment 8

Amano AK (30 mg) was added to a solution of racemic lupinine (500 mg, 2.95 mmol) in 5 ml of toluene and the mixture was stirred for 24 h. Benzyl acetate (1.6 ml, 10.9 mmol) was added and the mixture was stirred for 96 h at rt but the transesterification did not occur.

5.4. 7-Chloro-4-{*N*-[(1*RS*,9a*RS*)-octahydro-2*H*-quinolizin-1-yl)methyl]amino}-quinoline ((±)-AM1) and (+)-7-Chloro-4-{*N*-[(1*R*,9a*S*)-(octahydro-2*H*-quinolizin-1-yl)methyl]amino}-quinoline (+)-AM1. General method.

A mixture of (±)-aminolupinane [(1*RS*,9a*RS*)-octahydro-2H-quinolizine-1-methanamine], or (+)-aminolupinane ((1*R*, 9a*S*)-octahydro-2H-quinolizine-1-methanamine), prepared, in according to the method described by Sparatore F. et al,[25] starting from (±)-lupinine or (+)-lupinine (0.60 g, 3.57 mmol), 4,7-dichloroquinoline (0.71 g, 3.58 mmol) and phenol (2.3 g) was heated for 4 h at 180 °C under N_2. After cooling, the mixture was treated with 2 M NaOH and the mixture was extracted with DCM (3 times). The combined organic phases were dried and the solvent evaporated. The residue was purified by silica gel chromatography (CH_2Cl_2/CH_3OH/conc.NH_4OH; 90:9.7:0.3) and the solid crystallized from the indicated solvent.

5.4.1. 7-Chloro-4-{*N*-[(1*RS*,9a*RS*)-octahydro-2*H*-quinolizin-1-yl)methyl]amino}-quinoline ((±)-AM1)

Crystallized from abs. EtOH/Et_2O. Yield 55%; mp 168.5–170.6 °C. ^{1}H NMR ($CDCl_3$): δ 8.90 (s, 1H); 8.46 (d, J = 5.51 Hz, 1H); 7.99 (d, J = 1.92 Hz, 1H); 7.68 (d, J = 9.08 Hz, 1H); 7.35 (dd, J = 9.08, 2.20 Hz, 1H); 6.25 (d, J = 5.77 Hz, 1H); 3.63–3.57 (m, 1H); 3.38–3.32 (m, 1H); 3.07–3.03 (m, 2H); 2.25–1.15 (m, 14H). HRMS (ESI) m/z calcd for $C_{19}H_{25}N_3Cl$ $[M+H]^+$: 330.17315; found: 330.17268; calcd for $C_{19}H_{24}N_3ClNa$ $[M+Na]^+$: 352.15510; found: 352.15521.

5.4.2. (+)-7-Chloro-4-{*N*-[(1*R*,9a*S*)-(octahydro-2*H*-quinolizin-1-yl)methyl]amino}-quinoline (+)-AM1

Crystallized from CH_2Cl_2. Yield 48%; mp 147–149 °C. $[\alpha]_D^{20}$ +22.5 (c = 0.98 in EtOH). ^{1}H NMR ($CDCl_3$): δ 8.83 (s, 1H); 8.47 (d, J = 5.51 Hz, 1H); 7.97 (d, J = 1.93 Hz, 1H); 7.67 (d, J = 9.08 Hz, 1H); 7.34 (dd, J = 9.08, 2.20 Hz, 1H); 6.24 (d, J = 5.78 Hz, 1H); 3.65–3.50 (m, 1H); 3.40–3.20 (m, 1H); 3.10–2.95 (m, 2H); 2.30–1.00 (m, 14H); superimposable with a ^{1}H NMR spectrum of pure sample of (−)-AM1 (mp 142–144 °C; $[\alpha]_D^{20}$ −27.8; c = 1 in EtOH). HRMS (ESI) m/z calcd for $C_{19}H_{25}N_3Cl$ $[M+H]^+$: 330.17315; found: 330.17264; calcd for $C_{19}H_{24}N_3ClNa$ $[M+Na]^+$: 352.15510; found: 352.15544.

5.5. Parasite cultures and drug susceptibility assay

Plasmodium falciparum cultures were carried out according to Trager and Jensen with slight modifications.[26] The CQ-sensitive, strains D-10 and 3D7 and the CQ-resistant, strain W-2 and the Pf-Kenia isolate[27] were maintained at 5% hematocrit (human type A-positive red blood cells) in RPMI 1640 (EuroClone, Celbio) medium with the addition of 1% AlbuMax (Invitrogen, Milan, Italy), 0.01% hypoxantine, 20 mM Hepes, and 2 mM glutammine. The Pf Kenia isolate derives from a patient with malaria returning from Kenia to Milan and adapted to grow in culture. All the cultures were maintained at 37 °C in a standard gas mixture consisting of 1% O_2, 5% CO_2, and 94% N_2. Compounds were dissolved in either water or DMSO and then diluted with medium to achieve the required concentrations (final DMSO concentration <1%, which is non-toxic to the parasite). Drugs were placed in 96-well flat-bottomed microplates (COSTAR) and serial dilutions made. Asynchronous cultures with parasitaemia of 1–1.5% and 1% final hematocrit were aliquoted into the plates and incubated for 72 h at 37 °C. Parasite growth was determined spectrophotometrically (OD650) by measuring the activity of the parasite lactate dehydrogenase (pLDH), according to a modified version of the method of Makler in control and drug-treated cultures.[23] The antimalarial activity is expressed as 50% inhibitory concentrations (IC_{50}); each IC_{50} value is the mean and standard deviation of at least three separate experiments performed in duplicate.[7]

5.6. Cell toxicity assays

The long-term human microvascular endothelial cell line (HMEC-1) immortalized by SV 40 large T antigen[28] was maintained in MCDB 131 medium (Invitrogen, Milan, Italy) supplemented with 10% fetal calf serum (HyClone, Celbio, Milan, Italy), 10 ng/ml of epidermal growth factor (Chemicon), 1 μg/ml of hydrocortisone, 2 mM glutamine, 100 U/ml of penicillin, 100 l μg/ml of streptomycin, and 20 mM Hepes buffer (EuroClone). Human dermal fibroblasts (HDF) were maintained in DMEM medium (EuroClone) supplemented with 10% fetal calf serum, 2 mM glutamine, 100 units/ml of penicillin, 100 μg/ml of streptomycin. Unless stated otherwise, all reagents were from Sigma Italia, Milan, Italy. For the cytotoxicity assays, cells were treated with serial dilutions of test compounds and cell proliferation evaluated using the MTT assay already described.[24] Plates were incubated for 72 h at 37 °C in 5% CO_2, then 20 μL of a 5 mg/ml solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (M-2128 Sigma) in PBS was added for an additional 3 h at 37 °C. The plates were

then centrifuged, the supernatants discarded and the dark blue formazan crystals dissolved using 100 μL of lysing buffer consisting of 20% (w/v) of a solution of SDS (Sigma), 40% of *N*,*N*-dimethylformamide (Merck) in H_2O, at pH 4.7 adjusted with 80% acetic acid. The plates were then read on a microplate reader (Molecular Devices Co., Menlo Park, CA, USA) at a test wavelength of 550 nm and a reference wavelength of 650 nm. The results are expressed as IC_{50}, which is the dose of compound necessary to inhibit cell growth by 50%. All the tests were performed in triplicate at least three times.

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