Design, Synthesis and Antimalarial Activity of Trifluoromethylartemisinin–Mefloquine Dual Molecules

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Dedicated to Professor Iwao Ojima on the occasion of his 60th birthday

Malaria is one of the 'major parasitic diseases in many tropical and subtropical regions, causing more than one million deaths (principally among young African children) out of 600 million clinical cases each year. Today approximately 40% of the world's population live in areas where they are at risk of malaria infection.[1] During recent years, the situation has worsened, as malaria is undergoing a resurgence. One of the main obstacles to malaria control is the emergence and spread of strains of *Plasmodium falciparum* resistant to all first-line therapies (i.e. drugs belonging to either the quinoline or the antifolate groups).

Currently, in the absence of an effective malaria vaccine,[2] two approaches should be followed to deal with the spread of drug resistance in *P. falciparum*: i) the optimisation and rationalisation of the use of existing antimalarials by their administration in combination;[3] ii) the development of effective, easy-to-use and affordable new antimalarial drugs.[4]

Peters et al.[5] were the first to show that the bi-therapy strategy could be applied to the treatment of malaria, and that a judicious combination of antimalarial drugs could delay the selection of resistant mutants in vitro. However, all earlier examples of combination therapy in malaria failed to prevent the emergence of resistant strains of parasites.[3a] These failures were attributed either to the drugs used having similar mode of action or to resistance already having been developed to them. Thankfully, the appearance of a new class of drugs, artemisinin derivatives, in the "therapeutic arsenal" against malaria[6] allows new possibilities of combination therapy. Indeed, not only are artemisinin and its derivatives the fastest-acting antimalarials, but their tolerance and safety have been amply reported and their mode of action is unrelated to that of any other antimalarials.[7] To date, they remain the only class of drug to which *P. falciparum* has not become resistant in vivo.[6,8] However, because of their short in vivo half lives, their use as a mono-therapy has led to an high rate of recrudescent parasitemia after a short treatment. The underlying science behind the therapeutic effectiveness of artemisinin-based combination therapy (ACT) is that the artemisinin derivative, due to its gametocytocidal effect, rapidly kills most of the parasites; those that remain are then killed by a high concentration of the longer half-life partner drug. In this way, the probability that mutant parasites survive and emerge from these two drugs is very low.[9]

Since April 2001,[8] the World Health Organization has strongly recommended the use of ACT for the treatment of malaria in countries where there is resistance to conventional drugs, despite the fact that problems of adherence to nonfixed combinations and their rational use, particularly in the home, remain their major drawback. To date, amongst the recommended ACTs (artemether + lumefantrine, artesunate + amodiaquine, sulfadoxine–pyrimethamine or mefloquine), only the first is currently available as a fixed combination produced to Standards of Good Manufacturing Practice (COARTEM).[10] In fact, the combination most prescribed in areas of highest parasite resistance is artesunate + mefloquine.[11] Various studies conducted in south-east Asia highlighted the remarkable double effect of this last combination: a reduction of transmission from the host to the vector and the mutual protection of the drugs against resistance.[12]

In our program devoted to the synthesis of new antimalarial fluorinated artemisinin derivatives,[13] we have already shown that 10-trifluoromethyl artemisinin derivatives (CF_3-artemisinin derivatives)[13b] are more active and metabolically more stable than artesunate or artemether. We then continued our studies by designing a molecular hybrid in which the two active principles, one fluorinated artemisinin derivative and the mefloquine, are covalently bound. We expected that this new "double-drug" or dual molecule would reduce the risk of drug resistance by the mutual protection of each moiety and would be easy to use. This "covalent bi-therapy" approach seems to

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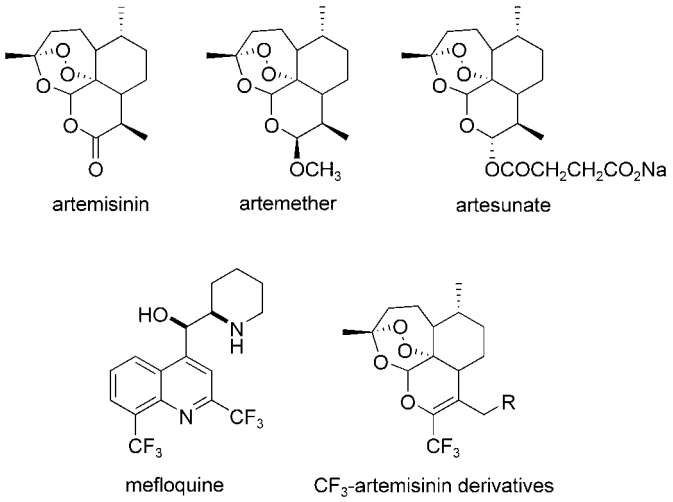
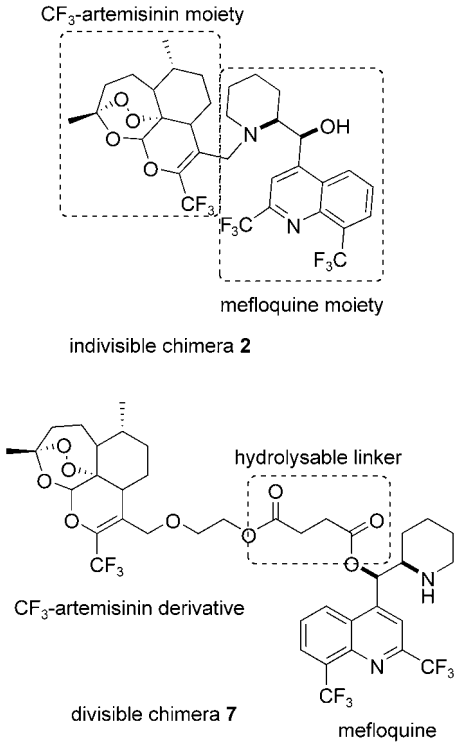




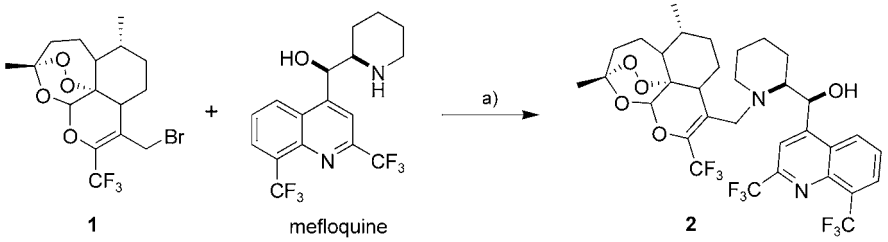
be promising for the treatment of malaria, as demonstrated with the recent reports of the development of trioxaquine, a chimeric molecule of a synthetic endoperoxide and the chloroquine,[14] and of primaquine-statine based derivatives.[15]

Taking advantage of the easy preparation of 16-functionalized CF_3-artemisinin derivatives,[13b,16] we planned to synthesized two types of CF_3-artemisinin–mefloquine dual derivatives. In the first case, the artemisinin moiety is linked to the mefloquine by a covalent, indivisible bond. The 4-quinolinemethanol structure will be retained because the alcohol function is indispensable for antimalarial activity.[17] In the second case, the artemisinin derivative and the mefloquine are bound via a diester linker, which should be easily hydrolysed by esterase(s) to allow the liberation in vivo of both active drugs. We now report the synthesis and the first biological data regarding these two CF_3-artemisinin–mefloquine derivatives.

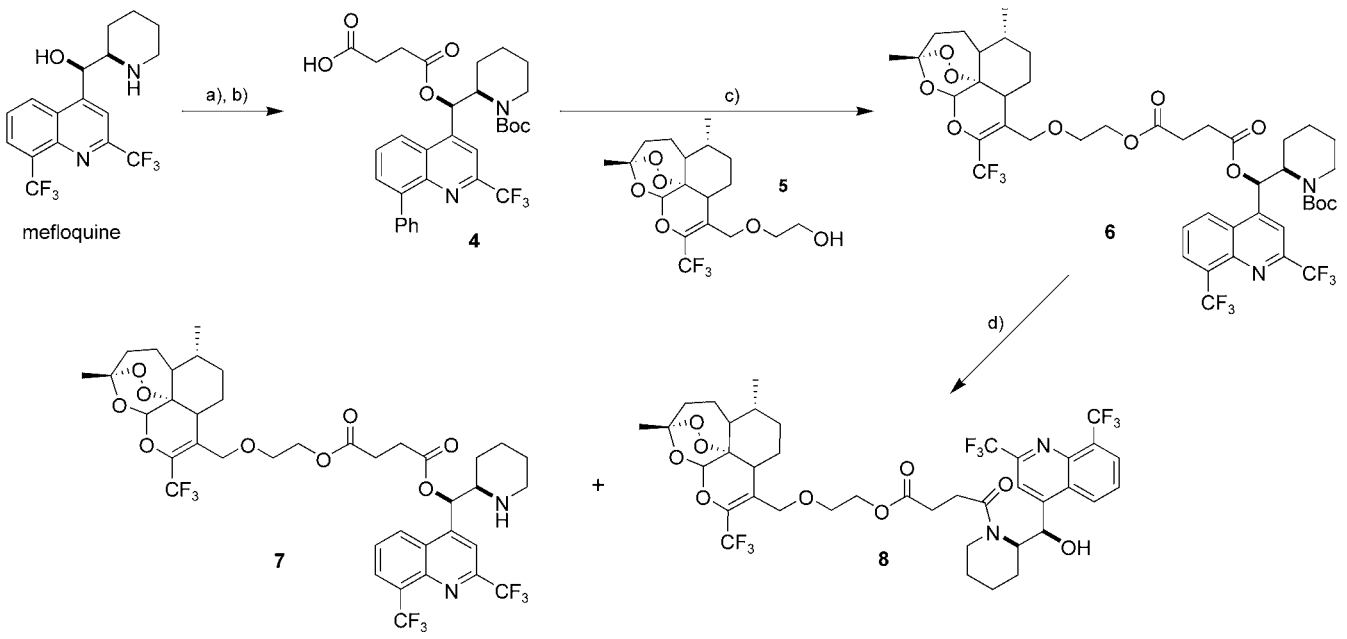
The synthesis of the indivisible chimera **2** was based on the nucleophilic substitution of the 10-CF_3 allylic bromide **1**[16] by the mefloquine in presence of Et_3N (Scheme 1). As we had previously noticed,[13b,16] under these conditions, the alcohol functions are unreactive, and competitive substitution by the hydroxyl group was thus excluded. After 4 days at room temperature, 90% of the starting materials were converted into chimera **2** (reaction monitored by ^{19}F NMR), which was then isolated in 61% yield.

The preparation of the divisible chimera **7** involved an esterification reaction between the acid **4** and the hydroxyether artemisinin derivative **5**, the antimalarial activity of which has already been proved.[13b] The piperidinyl amine of the mefloquine was first protected with a Boc group, and the linker was then introduced by treatment of the *N*-Boc mefloquine with succinic anhydride and Et_3N in refluxing chloroform (Scheme 2). Esterification of the resulting acid **4** with the hydroxyether artemisinin derivative **5**,[13b] by using dicyclohexyl carbodiimide (DCC) in presence of a catalytic amount of 3,4-dimethylamino pyridine (DMAP), afforded the protected chimera **6**. Finally, deprotection of the piperidinyl amine with trifluoroacetic acid (TFA) led to the divisible chimera **7**, which was isolated in 57% yield (Scheme 2). During the course of this reaction, another minor product, **8**, was formed and isolated in 25% yield. Despite the complexity of the ^1H and ^{13}C NMR spectra of **8**, an observed deshielded signal for one of the carbonyl functions (δ_{CO} = 172.0 and *173.0* ppm, instead of *171.4* and 172.0 ppm for the diester **7**) prompted us to postulate that **8** could be an amidoester, the product of the intramolecular rearrangement of **7** under acidic conditions. The structure of **8** was confirmed by comparison of its NMR data with those of a simplified amidoester model **11** (δ_{CO} = 172.0 and *173.8* ppm), prepared by an unambiguous route (Scheme 3).

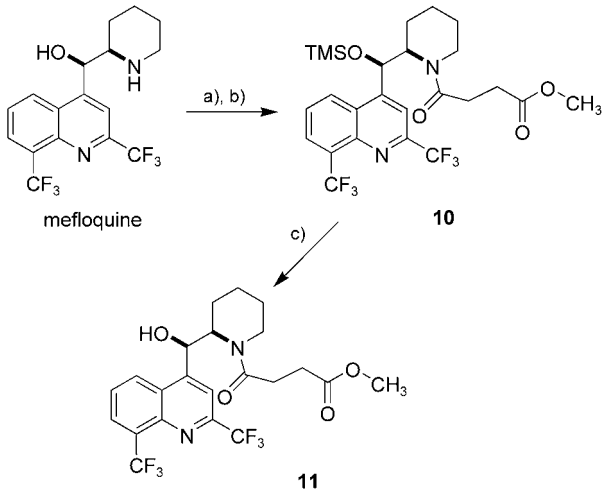
Chimeras **2** and **7** were tested in vitro against four strains of *P. falciparum* that showed different degrees of resistance to mefloquine (F32 > Thai > FcB1 > K1, F32 being the most resistant) or chloroquine (K1 > FcB1 > F32 > Thai, K1 being the most resistant). The inhibition concentrations able to reduce the parasitemia by 50% within 48 h (IC_{50}) were determined as described by Desjardins et al.[18] The sus-



Scheme 1. *Preparation of the indivisible chimera* ***2****. Reagents and conditions: a)* Et_3N *(1 equiv), THF, RT, 4 days (61%).*



Scheme 2. *Preparation of the divisible chimera **7**. Reagents and conditions: a) Boc_2O (1.5 equiv), Et_3N (2.5 equiv), THF, 0 °C, 2 h (98 %); b) succinic anhydride (4 equiv), Et_3N (2.5 equiv), $CHCl_3$, reflux, 18 h (75 %); c) DCC (1.1 equiv), DMAP (0.1 equiv), 0 °C to RT, 24 h (71 %); d) TFA (4 equiv), CH_2Cl_2, RT, 24 h (**7**: 57 %, **8**: 25 %).*



Scheme 3. *Preparation of model **11**, a simplified analogue of **8**. Reagents and conditions: a) TMSCl (2 equiv), Et_3N (3 equiv), THF, 0 °C to RT, 18 h (90 %); b) methyl 4-chloro 4-oxobutyrate (2 equiv), pyridine (4 equiv), CH_2Cl_2, 0 °C to RT, 3 h (81 %); c) $Bu_4NF{\cdot}3H_2O$ (1.1 equiv), THF, RT, 24 h (76 %).*

Table 1. *In vitro antiplasmodial activity against four different strains of* P. falciparum *(IC_{50} values are the mean ± S.D. of three independent experiments).*

P. falciparum strain	IC_{50} [nM] F32	Thai	FcB1	K1
mefloquine	23.2 ± 2.3	15.5 ± 1.1	12.7 ± 1.5	2.8 ± 0.5
artemether	2.2 ± 0.3	2.3 ± 0.1	3.0 ± 0.8	1.5 ± 0.2
chloroquine	19 ± 4	14.3 ± 2.4	105 ± 16	183 ± 35
CF_3-artemisinin derivative **5**[13b]	–	–	3.7 ± 0.5	–
indivisible chimera **2**	15.7 ± 1.1	12.7 ± 3.9	17.2 ± 1.5	10.6 ± 0.2
divisible chimera **7**	6.6 ± 2.0	4.5 ± 0.5	5.4 ± 1.7	2.4 ± 0.4

ceptibility of these strains to mefloquine, chloroquine and artemether is indicated in Table 1. Compounds **2** and **7** were highly active against all strains in the low nanomolar range (IC_{50} values ranging from 2.4 to 17.2 nM). No significant difference of sensitivity to chimera **2** was observed between the different strains; this indicated the absence of cross-resistance with chloroquine and mefloquine. However, chimera **2** is slightly less efficient at inhibiting parasite growth than artemether alone and than the chimera **7**. In contrast, a slight difference of sensitivity was observed for the divisible chimera **7** between the different strains (IC_{50} in a ratio from 1 to 3). This difference of sensitivity to chimera **7** correlates with the difference in sensitivity between each strain to mefloquine and suggests: i) an efflux of the chimera **7** from the parasite. Polymorphisms in Pfmdr1, the gene encoding the *P*-glycoprotein homologue 1 (Pgh1) protein, have been implicated in resistance to mefloquine and act as an efflux pump;[19] or ii) an hydrolysis of the divisible chimera **7** by esterase(s) and an afflux of the mefloquine moiety from the parasite. For the former hypothesis, the better efficiency of the chimera **7** compared to mefloquine to inhibit the growth of mefloquine-resistant strains might result mainly from the activity of the CF_3-artemisinin moiety.

The potentials of the indivisible chimera **2** and of the divisible chimera **7** as antimalarial drugs were then confirmed in vivo in mice according to Peters' protocol.[20] Both chimeras **2** and **7** and the CF_3-artemisinin derivative **5**,[13b] precursor of the divisible chimera, were studied in vivo in groups of five mice

infected with the murine *Plasmodium berghei* strain NK173 (intraperitoneal administration, at a dose of 35.5 μmol kg^{-1}, strain nonresistant to mefloquine; Figure 1). At this concentration,

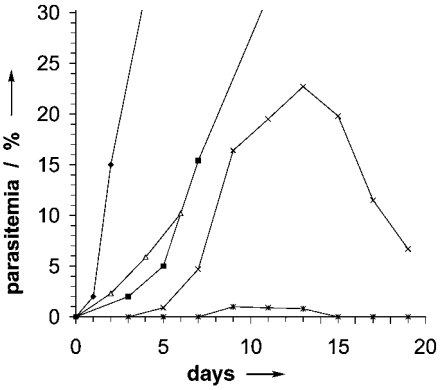


Figure 1. *In vivo antimalarial activity on mice infected by* P. berghei *NK 173. In vivo assays were performed according to Peters' protocol[20] with the fixed concentration of drug at 35.5 μmol kg^{-1} per injection. ◆: control, ■: artemether, △: CF_3-artemisinin derivative **5**, ×: indivisible chimera **2**, *: divisible chimera **7**.*

both chimeras **2** and **7** were more efficient in controlling the parasitemia than the reference drug, artemether. During treatment with the indivisible chimera **2**, the parasitemia increased less rapidly than after treatment with artemether (at day 5, chimera **2** was already five times more efficient at inhibiting parasite growth than artemether), and after the 13th day the parasitemia was controlled by mice and decreased. Remarkably, the divisible chimera **7** was highly effective against the parasite growth and was much more efficient than its precursor CF_3-artemisinin derivative **5**.[13b] Only a very slight parasitemia (< 1 %) could be detected between days 9 and 13. This result strengthens our hypothesis of hydrolysis in vivo of the diester linker of the divisible chimera **7** to liberate both **5** and mefloquine. As in ACT,[9] the parasites remaining after their exposure to the CF_3-artemisinin **5**, would be killed by the mefloquine.

We have designed and synthesised two new types of CF_3-artemisinin–mefloquine dual molecules. In the first case, the indivisible chimera **2**, the CF_3-artemisinin moiety was covalently linked to the piperidinyl amine of the mefloquine. In the second case, the divisible chimera **7**, the CF_3-artemisinin derivative was bound to the mefloquine via a diester linker, which was expected to be easily hydrolysed in vivo. In vitro, compounds **2** and **7** showed an efficacy against the four different strains of *Plasmodium falciparum*, which exhibited different degrees of resistance to mefloquine and chloroquine in the low nanomolar range (IC_{50} values ranging from 2.4 to 17.2 nM). In vivo, both chimera **7** and, to a lesser extent, chimera **2** were highly active, more efficient in inhibition of parasite growth than the reference drug, artemether. Moreover, these preliminary in vitro and in vivo biological results support the hypothesis we followed to design these dual antimalarial molecules, and are encouraging for the application of this approach to new compounds.

Experimental Section

In vivo and in vitro assays as well as experimental details about the preparation and spectroscopic characterisation of all new compounds (**2**–**4**, **6**–**11**) are described in the Supporting Information.

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