Preparation and Antimalarial Activities of "Trioxaquines", New Modular Molecules with a Trioxane Skeleton Linked to a 4-Aminoquinoline

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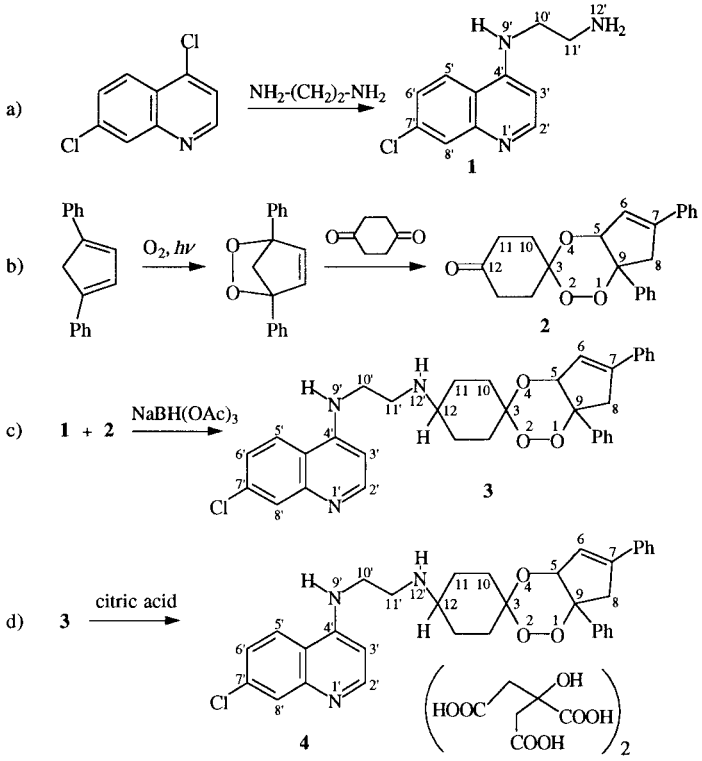
Malaria is the third-most cause of death by infectious diseases, after tuberculosis and AIDS. The estimates for the annual mortality are ranging from 1 to 2.5 million deaths.[1] Chloroquine (CQ) has been a cheap and efficient antimalarial drug for more than 40 years, but now most of the *Plasmodium falciparum* strains, responsible for severe malaria, are resistant to this classical drug. The multidrug-resistant form of malaria is widespread in Africa and in South-East Asia and continues to rise all around the world, not only because of the resistance of the parasite itself, but also due to the resistance of mosquitoes to cheap insecticides and to the possible consequence of the global warming on the extension of endemic malaria zones.[2]

The rational strategy to fight against drug-resistant diseases is to combine active molecules with independent modes of action to prevent the emergence of resistance. This strategy was first developed in antituberculosis chemotherapy, then in cancer chemotherapy, and more recently for the treatment of AIDS. In order to "roll back malaria", as demanded by the director of the WHO in 1998, new drugs should be discovered and polychemotherapy should be adopted as a regular therapy approach.[2] Based on our studies concerning the mechanism of action of antimalarial drugs related to artemisinin,[3] we decided to prepare new chimeric molecules by covalently attaching a trioxane motif to a 4-aminoquinoline moiety.[4] This concept of modular molecules combines a peroxide entity acting as a potential alkylating agent[3, 5] with an aminoquinoline entity that is known to easily penetrate into infected erythrocytes.[6] The pharmacological target of such modular (or dual) molecules, called "trioxaquines", will be the free heme liberated during the hemoglobin digestion by the schizonts within infected red blood cells. These modular molecules can be regarded as potential bitherapy drugs, that is, they contain two therapeutically active moieties combined within one molecule, and might be able to prevent drug resistance (in addition, it should be noted that for antimalarial endoperoxide drugs the resistance was lost once drug selection pressure had been withdrawn[7]).

Here we report the preparation of a series of trioxaquines and their biological activities on different *P. falciparum* strains. The convergent route used for the synthesis of trioxaquines is depicted in Scheme 1 (for obvious reasons, these modular molecules must be cheap to prepare and easily accessible). In the case of **4**, 4,7-dichloroquinoline was condensed with 1,2-diaminoethane to give the aminoquinoline derivative **1** in 42% yield (Scheme 1 a; reported yields have not been optimized). The trioxane-ketone **2** was prepared in 55% yield by reacting 1,4-cyclohexanedione with the endoperoxide obtained by photooxygenation of 1,4-diphenylcyclopenta-1,3-diene (Scheme 1 b). The reductive amination of **2** with the primary amine of the aminoquinoline **1** provided the trioxaquine **3** (**ODC-188** in Table 1) in 87% yield (Scheme 1 c). To enhance the solubility of this trioxaquine, the corresponding dicitrate derivative **4** (**ODC-218** in Table 1) was prepared (Scheme 1 d; NMR spectroscopic measurements in [D_6]DMSO confirmed that *N*12′ is the protonation site; in fact, the second citric acid molecule is not acting as a proton source).



Scheme 1. *Convergent synthesis of the trioxaquine **4**. Reagents and reaction conditions for steps a and b can be found in the Supporting Information.*

Four trioxaquine derivatives, **ODC-182**, **ODC-188**, **ODC-190**, and **ODC-218** (Figure 1), were tested against three different

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*Table 1. IC_{50} values for the biological activities of **ODC-182**, **ODC-188**, **ODC-190**, and **ODC-218** against three different strains of* P. falciparum.

Compound	FcB1-Colombia (CQR)[a] IC_{50} [ng mL^{-1}] ([nM])	FcM29-Cameroon (CQR+)[a] IC_{50} [ng mL^{-1}] ([nM])	Nigerian (CQS)[a] IC_{50} [ng mL^{-1}] ([nM])
ODC-190 ($n=3$)	35 (60)	10 (17)	50 (86)
ODC-18 ($n=4$)	10 (17)	20 (34)	20 (34)
ODC-188 ($n=2$)	5 (9)	10 (18)	1 (2)
ODC-218 ($n=2$, dicitrate)	20 (21)	n.d.[b]	7 (8)
Aminoquinoline **1**[c]	– (36)	n.d.[b]	– (45)
Trioxane ketone **2**[c]	n.d.[b]	n.d.[b]	– (28)
Chloroquine diphosphate[c]	60 (116)	80 (155)	10 (19)

[a] CQR = chloroquine(CQ)-resistant strain, CQR+ = highly chloroquine-resistant strain, CQS = chloroquine-sensitive strain. All values determined after 72 h. [b] n.d. = not determined. [c] Data indicated for comparison.

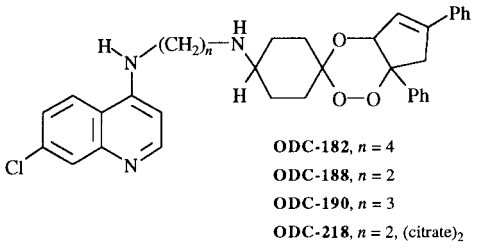

Figure 1. *Chemical structures of trioxaquines tested against* P. falciparum.

strains of *P. falciparum*: a Nigerian CQ-sensitive (CQS) strain, FcB1, and FcM29 (CQ-resistant and highly CQ-resistant, respectively). It should be noted that the different stereoisomers of each trioxaquine were not evaluated independently at the present stage of this work. The inhibition concentrations able to reduce the parasitemia by 50% within 72 h (IC_{50} values) were determined on infected human red blood cells according to refs. [8], [9] (Table 1).

All IC_{50} values obtained for the different trioxaquines are ranging from 2 to 86 nM. On the nigerian CQS strain, IC_{50} values are modulated by the length of the linker between the trioxane motif and the aminoquinoline entity, a short tether ($n=2$) being better than the longer ones ($n=3$ or 4). The dicitrate trioxaquine **ODC-218** or its base-analogue **ODC-188** are more active on the different strains of *Plasmodium falciparum* than chloroquine itself and than each precursor (see data on the nigerian strain), indicating that the covalent attachment of both precursors, aminoquinoline **1** and trioxane-ketone **2**, is required to obtain a synergic effect between the two different constituents of the dual molecule. These trioxaquine derivatives are highly active on chloroquine-resistant strains (IC_{50} values at 72 h being 9 and 21 nM for **ODC-188** and **ODC-218**, respectively, compared to 116 nM for chloroquine itself). The present data valid the choice of our strategy for the design of new antimalarial drugs based on modular molecules.

In the future development of the present work we will consider the design of trioxaquines with different types of linkers, with also modifications of the trioxane and the quinoline motifs.

Experimental Section

The preparation of N^2-(7-chloro-4-quinolinyl)-1,2-diaminoethane (**1**) and trioxane-ketone **2** (steps a and b,[10] respectively, Scheme 1) are available as Supplementary Material.

Trioxaquine 3 (ODC-188) (step c, Scheme 1):[11] Trioxane-ketone **2** (99 mg, 0.27 mmol) and aminoquinoline **1** (76 mg, 0.34 mmol) (molar ratio **1**:**2** = 1.25) were mixed in dichloromethane (5 mL) before addition of sodium triacetoxyborohydride (72 mg, 0.34 mmol). The reaction was stirred at room temperature for 18 h and then washed with distilled water. The organic layer was dried and evaporated under vacuum to dryness (crude yield = 87%). ^{1}H NMR (250 MHz, $CDCl_3$, TMS): $\delta = 8.50$ (2 × d, 1 H; H2′), 7.95 (2 × d, 1 H; H8′), 7.70 (2 × d, 1 H; H5′), 7.63 – 7.25 (m, 11 H; H6′ and 10 H-phenyl), 6.35 (m, 2 H; H3′ and H6), 5.99 (br. s, 1 H; *H*N9′), 5.17 (2 × br. s, 1 H; H5), 3.31 (m, 3 H; H_2C10′ and *H*C8), 3.05 (m, 3 H; H_2C11′ and *H*C8), 2.61 (m, 2 H; cyclohexyl), 2.42 (m, 1 H; *H*C12), 2.10 – 1.25 (6 H; cyclohexyl, and 1 H, *H*N12′); ^{1}H NMR (250 MHz, [D_6]DMSO, TMS): $\delta =$ 8.51 (2 × d, 1 H; H2′), 8.37 (2 × d, 1 H; H5′), 7.89 (2 × d, 1 H; H8′), 7.75 – 7.35 (m, 11 H; H6′ and 10 H-phenyl), 7.38 (br. s, 1 H; *H*N9′), 6.61 (m, 2 H; H3′ and H6), 5.38 (2 × br. s, 1 H; H5), 3.40 (m, 2 H; H_2C10′), 3.27 (d, 1 H; *H*C8), 3.08 (d, 1 H; *H*C8), 2.92 (q, 2 H; H_2C11′), 2.70 – 2.40 (m, 3 H; *H*C12 and 2 H cyclohexyl), 1.95 – 1.25 (m, 6 H; cyclohexyl); MS (DCI, NH_3^+): *m/z* (%): 566 (11), 568 (100) $[M+H]^+$, 569 (38), 570 (41), 571 (12). Trioxaquines **ODC-182** and **ODC-190** were prepared according the protocols described above for **ODC-188**. NMR and MS data were consistent with the structures.

Trioxaquine dicitrate 4 (ODC-218): The trioxaquine **3** (25 mg, 0.04 mmol) was solubilized in acetone (0.5 mL) before adding a solution of citric acid (17 mg, 2 equiv) in 0.5 mL of acetone. The trioxaquine dicitrate precipitated spontaneously. After centrifugation, the precipitate was washed with diethyl ether and dried under pump vacuum (quantitative yield). ^{1}H NMR (250 MHz, [D_6]DMSO, TMS): $\delta = 8.60$ (2 × d, 1 H; H2′), 8.35 (2 × d, 1 H; H5′), 7.95 (2 × d, 1 H; H8′), 7.70 (m, 5 H; H6′ and 4H-phenyl), 7.50 (m, 6 H; phenyl), 6.73 (2 × d, 1 H; H3′), 6.60 (2 × q, 1 H; H6), 5.42 (2 × br. s, 1 H; H5), 3.71 (m, 2 H; H_2C10′), 3.55 – 3.25 (m, 4 H; H_2C11′, *H*C8 and *H*C12), 3.12 (d, 1 H; *H*C8), 2.76 (d, 4 H; citrate), 2.65 (d, 4 H; citrate), 2.60 (m, 1 H; cyclohexyl), 2.10 – 1.50 (7 H; cyclohexyl); MS (ESI, positive mode): *m/z*: 568.2 $[M]^+$, *M* corresponding to the protonated base compound; (ESI, negative mode): *m/z*: 190.9 [citrate].

Determination of biological activities: *P. falciparum* were cultivated in continuous culture according to the method of Trager and Jensen,[8] with the following modifications:[9] Parasites were maintained within human red blood cells (O±), diluted at 1% of hematocrite in a RPMI 1640 mixture (BioMedia, Boussens, France) supplemented with 25 mM Hepes and 30 mM $NaHCO_3$ and complemented with 5% of human serum AB+. Parasite population were synchronized over a period of 4 h with a solution of gelatin and then by lysis with D-sorbitol at 5%.[12, 13] The nigerian strain was considered as being chloroquine-sensitive and both strains FcM29-Cameroon

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and FcB1-Columbia are chloroquine-resistant (CI_{50} of chloroquine > 100 nM).[14, 15] Biological activities were measured by the method of Desjardins et al.[16] Assays were performed in triplicate in 96-microplates, cultures being at 1% of hematocrite and with 0.5 – 1% of parasitemia.

For each assay, parasites were incubated with decreasing concentrations of drugs during 32 h (data not shown) or 72 h (four wells contained chloroquine as reference). The initial drug dilutions were made at 10 mg mL^{-1} in dimethylsulfoxide and the next ones with RPMI 1640. The parasite growth was measured by incorporation of tritiated hypoxanthine and compared with the incorporation in the absence of drug (used as 100% value).[17] IC_{50} values were determined by tracing the percentage of inhibition as function of the drug concentration. The IC_{50} values measured at 32 h correspond to the action of the drug on the trophozoite stage and IC_{50} values measured at 72 h (time for 1.5 parasite life cycles) indicate a possible effect on the liberation and reinvasion steps of the erythrocytes by the parasites.

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Designed Prostaglandins with Neurotrophic Activities

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Cyclopentenone prostaglandins (PGs), such as PGA_2 and Δ^{12}-PGJ_2, are recognized as important biosignaling molecules. They participate in cell growth regulation, cell differentiation, and inflammation processes by modulating the expression of a variety of genes and the function of proteins, according to the structures and the cell types in which the latter are found.[1] We previously demonstrated that Δ^7-PGA_1 methyl ester (**1**) and its analogues suppress the growth of glioma cells at the G1 phase of the cell cycle by inducing the expression of cyclin-dependent kinase inhibitor p21 and reducing cyclin E expression.[2] Interestingly, we observed that some Δ^7-PGA_1 methyl ester derivatives caused morphological changes as well as growth arrest in glioma cells, which are typical brain tumor cells, during the study of the antiproliferative effects of these compounds;[3] this suggests that the cells were differentiated by the PGs. In addition, oligomers of PGB_1 derivatives reportedly exhibited neuroprotective activities in cerebral ischemia.[4] However, there has been little information on the actions of structurally defined cyclopentenone PGs in neuronal cells in spite of the abundant distribution of their precursors and PG synthases in the brain.[5] We have been intrigued with examining the effects of cyclopentenone PGs on a standard neuronal cell line, and recently found that natural Δ^{12}-PGJ_2 and its dehydrated derivative 15-deoxy-$\Delta^{12,14}$-PGJ_2 (**2**), which possess a cross-conjugated dienone structure, promoted neurite outgrowth in the presence of nerve growth factor (NGF) in PC12 cells.[6] Based on the extensive study of these natural PGs, we describe here the successful design and

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