In Vitro Activities of DU-1102, a New Trioxaquine Derivative, against *Plasmodium falciparum* Isolates

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The antimalarial trioxaquine derivative DU-1102, synthesized by covalent linkage between aminoquinoline and trioxane moieties, was highly active against Cameroonian isolates (mean 50% inhibitory concentration of 43 nmol/liter) of *Plasmodium falciparum*. There was no correlation between the responses to DU-1102 and chloroquine and only a low correlation between the responses to DU-1102 and pyrimethamine, suggesting an independent mode of action of the trioxaquine against the parasites.

Because of the extensive spread of drug resistance involving primarily 4-aminoquinolines and antifolate drugs in most of the geographic areas where *Plasmodium falciparum* is endemic, there is a need for a sustained search for promising compounds with new chemical structures and mechanisms of action. In the past 2 decades, only a few compounds belonging to a new class of antimalarial drugs, including aminoalcohols (mefloquine, halofantrine, lumefantrine), sesquiterpene trioxanes (artemisinin derivatives), and naphthoquinones (atovaquone), were developed for commercialization.

DU-1102 (Fig. 1a) is a new candidate compound that belongs to a novel chemical class named trioxaquine (7, 11) (Fig. 1b). Trioxaquines are new modular molecules obtained by covalently attaching a trioxane motif, present in artemisinin, to a 4-aminoquinoline entity, contained in chloroquine. As the trioxane moiety is a potential alkylating agent after reductive activation by heme (6, 10, 15–18) and the 4-aminoquinoline entity is known to easily penetrate within infected erythrocytes (9) and then interact with heme, such modular molecules are expected to combine the properties of both fragments. Although it remains to be experimentally confirmed, we hypothesized that trioxaquine is able to penetrate infected red blood cells and then interact with the free heme liberated during the hemoglobin digestion.

The in vitro activity of DU-1102 was assessed against clinical isolates of *P. falciparum* obtained in Yaoundé, Cameroon, where previous in vitro and in vivo studies have shown a high proportion (>40%) of chloroquine and/or pyrimethamine resistance (3, 14). We also evaluated the potential for in vitro cross-resistance between DU-1102 and chloroquine and pyrimethamine, which are first- and second-line (pyrimethamine-sulfadoxine combination) drugs in Cameroon, respectively.

Fresh clinical isolates of *P. falciparum* were obtained before treatment from adult Cameroonian patients attending the Nlongkak Catholic missionary dispensary in Yaoundé in 2000.

The patients presented with signs and symptoms of acute uncomplicated malaria and had at least 0.1% asexual parasitemia. The Saker-Solomons colorimetric test for 4-aminoquinolines, quinine, and antifolate drugs in urine was negative for each patient (12). The patients were treated with oral amodiaquine, according to the national antimalarial guidelines set by the Cameroonian Ministry of Public Health. The study was approved by the Cameroonian National Ethics Committee and the Cameroonian Ministry of Public Health.

Chloroquine sulfate was obtained from Rhône-Poulenc-Rorer (Antony, France). Pyrimethamine base was obtained from Sigma Chemical Company (St. Louis, Mo.). The procedures involved in the chemical synthesis of DU-1102 (molecular weight = 952.4) were recently reported (7) (it should be noted that DU-1102 is a new serial number of the trioxaquine derivative named ODC218 in reference 7). Stock solutions of chloroquine, pyrimethamine, and DU-1102 were prepared in sterile distilled water, absolute ethanol, and dimethyl sulfoxide, respectively. Twofold serial dilutions of chloroquine (final concentration, 25 to 1,600 nmol/liter) and DU-1102 (final concentration, 3.8 to 484 nmol/liter) and fourfold dilutions of pyrimethamine (final concentration, 0.05 to 51,200 nmol/liter) were prepared in sterile distilled water. The final concentration of dimethyl sulfoxide was <0.1%, which is nontoxic for parasite growth. Each concentration was distributed in triplicate in 96-well tissue culture plates. The isotopic microtest developed by Desjardins et al. was used in this study (2, 8). The suspension of infected erythrocytes (200 μl) was distributed in each well of the 96-well tissue culture plates. The parasites were incubated at 37°C in 5% CO_2 for 18 h. To assess parasite growth, [^{3}H]hypoxanthine (specific activity, 16.3 Ci/mmol; 1 μCi/well; Amersham, Little Chalfont, Buckinghamshire, United Kingdom) was added. After an additional 24 h of incubation (48 h for pyrimethamine and DU-1102), the plates were frozen to terminate the in vitro assay. The incorporation of [^{3}H]hypoxanthine was quantitated using a liquid scintillation counter, and the 50% inhibitory concentrations (IC_{50}s) were determined by nonlinear regression analysis.

The threshold IC_{50}s for in vitro resistance to chloroquine and pyrimethamine were estimated to be >100 nmol/liter (3,

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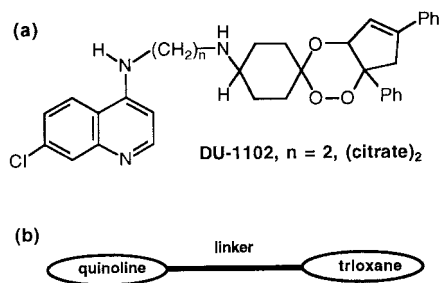
FIG. 1. (a) Chemical structure of DU-1102; (b) general structure of trioxaquines.

14). Data were expressed as geometric mean IC_{50}s. The mean IC_{50}s of DU-1102 for the chloroquine-sensitive and the chloroquine-resistant isolates were compared using the unpaired *t* test. Correlation of the IC_{50}s of different drugs was calculated by Spearman rank correlation. The significance level was fixed at 0.05.

The in vitro activity of DU-1102 against 32 *P. falciparum* isolates was assessed. Thirteen isolates (41%) were chloroquine sensitive (mean $IC_{50} = 36.0$ nmol/liter; range, 18.6 to 90.0 nmol/liter), and 19 (59%) were chloroquine resistant (mean $IC_{50} = 227$ nmol/liter; range, 106 to 390 nmol/liter) (Fig. 2). Thirty of 32 isolates developed adequately in *p*-aminobenzoic acid- and folic acid-free RPMI 1640 culture medium used to determine the in vitro activity of pyrimethamine. Six isolates (20%) were pyrimethamine sensitive (geometric mean $IC_{50} = 0.068$ nmol/liter; range, 0.011 to 84.3 nmol/liter), and 24 (80%) were pyrimethamine resistant (geometric mean $IC_{50} = 922$ nmol/liter; range, 136 to 26,000 nmol/liter). The geometric mean IC_{50} of DU-1102 was 43.1 nmol/liter (range, 11.2 to 71.0

nmol/liter). There was no significant difference ($P > 0.05$) between the mean IC_{50}s of DU-1102 for chloroquine-sensitive isolates (48 nmol/liter) and chloroquine-resistant isolates (40 nmol/liter). Moreover, the IC_{50}s of DU-1102 were not correlated with those of chloroquine (coefficient of correlation [*r*] = −0.140; *n* = 32; *P* > 0.05). The correlation coefficient between the IC_{50}s of DU-1102 and those of pyrimethamine was low (−0.439 for pyrimethamine-resistant isolates; *P* < 0.05). This result seems to suggest a low but existing probability of cross-resistance between pyrimethamine and DU-1102. In fact, because 80% of the isolates used in this study were pyrimethamine resistant, the low correlation between the responses to pyrimethamine and DU-1102 is most likely due to the skewed distribution of resistant parasites.

A previous study on other trioxaquine derivatives has shown that these compounds are highly active against chloroquine-sensitive and chloroquine-resistant reference clones of *P. falciparum*, with IC_{50}s of <90 nmol/liter and in most cases of <40 nmol/liter (7). The trioxaquine derivative DU-1102 used in the present study is in fact the molecule which has been considered, up to now, the most active when using reference clones of *P. falciparum*. This trioxaquine has citrate as a counterion, and the linker between the trioxane and the aminoquinoline structure contains two methylene groups (Fig. 1a).

Combination in antimalarial therapy is advocated by many malaria researchers as one of the means to fight against the spread of resistance to classic and new drugs (13, 19). By analogy with this therapeutic approach, trioxaquines have been prepared by linking two active pharmacophores. The quinoline nucleus has been a chemical reference of highly active antimalarial drugs for many decades, and several effective drugs containing this entity (chloroquine, amodiaquine, primaquine,
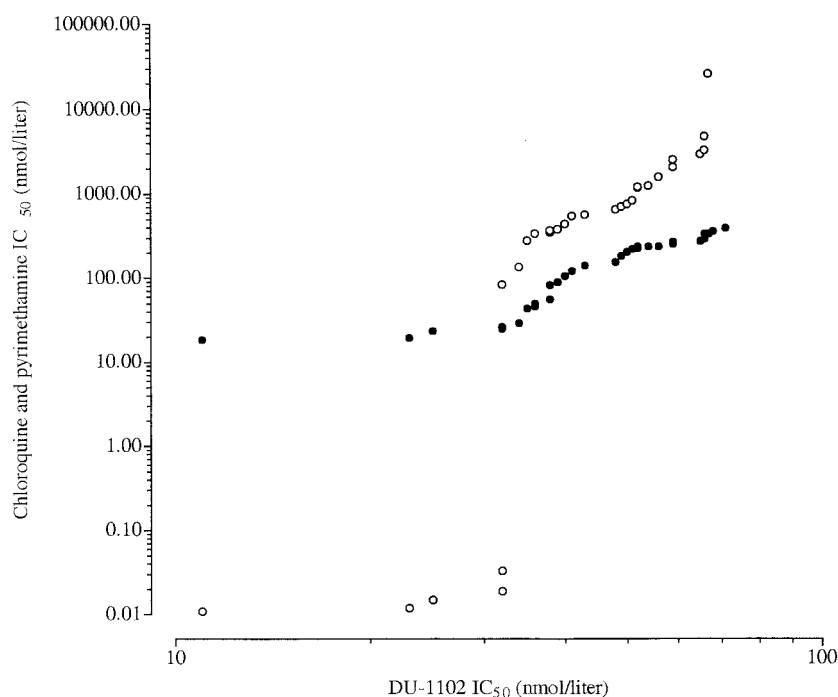

FIG. 2. Scatterplots showing the in vitro activities of DU-1102 versus chloroquine (black circles) and pyrimethamine (open circles) against Cameroonian clinical isolates of *P. falciparum*.

quinine, and mefloquine) have been developed. Trioxane contains a peroxide bridge which has been shown to be essential for the high activity of artemisisnin (1). The reductive activation of artemisinin and other endoperoxide-containing drugs by Fe^{II}-heme generates alkylating C-centered radicals (4, 5, 10, 15). The derivatives containing trioxane moiety, including artemether, arteether, dihydroartemisinin, and artesunate, are currently used as efficient and rapidly acting antimalarial drugs (18). Although the exact mechanism of action of trioxaquines is still under investigation, the available in vitro data indicate promising results for further development of this new class of antimalarial drugs based on a modular molecule strategy.

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