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From a cytotoxic agent to the discovery of a novel antimalarial agent

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ABSTRACT

A novel cytotoxin 3,5-bis(4-chlorobenzylidene)-1-[4-{2-(4-morpholinyl)ethoxy}phenyl-carbonyl]-4-piperidone hydrochloride **2** demonstrated potent antimalarial properties with IC_{50} values of 0.60 and 1.97 μM against the drug sensitive D6 strain and the C235 drug-resistant strain of *Plasmodium falciparum*. This compound concentrates in red blood cells, lowers glutathione concentrations in erythrocytes and permeates across CACO-2 cells. These data reveal **2** to be a promising lead compound in the quest for novel antimalarial agents.

In 2010, an estimated 781,000 deaths were attributed to malaria and currently well over 200 million people are affected by this disease.[1] The most severe cases of malaria are caused by *Plasmodium falciparum*[2] and isolates displaying multidrug resistance (MDR) have emerged.[3,4] There is an urgent need therefore to discover novel agents to treat malaria and especially to eradicate those parasites which display MDR.

For many years, the main interest of one of these laboratories is the development of different clusters of candidate cytotoxic agents and in particular compounds containing the 1,5-diaryl-3-oxo-1,4-pentadienyl pharmacophore.[5] A recent patent has been filed on series **1** many of which are potent cytotoxins and have MDR-revertant properties (Fig. 1).[6] The compounds in series **1** were designed as thiol alkylators[7] and it is of interest to note that depletion of glutathione (GSH) in drug-resistant strains of *P. falciparum* may restore drug sensitivity.[8] In particular, **2** emerged from this study as a potent cytotoxin (Fig. 1). For example, the IC_{50} values of **2** towards HSC-2998 and SW-620 human colon cancer cells are <0.005 and 1.45 μM, respectively.[7] Subsequent studies revealed that **2** had greater lethal effects towards a number of malignant cell lines than normal cells[9] and it possessed MDR-revertant properties.[10] In addition, this compound was well tolerated in mice.[11] There is a growing interest to evaluate anticancer drugs as candidate antimalarial agents for two reasons at least. First, there is evidence that certain anticancer drugs are effective as antimalarial agents. For example, various antineoplastic antimetabolites are active against drug-sensitive and drug-resistant isolates of *P. falciparum*.[12] Second, while many pharmaceutical companies may be reluctant to invest in attempts to find new antimalarials,[13] the finding of anticancer drugs (where the toxicology, dose levels and tolerance, for example, are known) which are lethal to *P. falciparum* will enable their rapid approval for antimalarial therapy. Based on the above considerations, the objective of the present investigation was to examine the potential of a potent cytotoxic agent **2** as a novel candidate antimalarial agent.

The synthesis of **2** has been described previously.[7] In brief, methyl 4-hydroxybenzoate was reacted with 2-(4-morpholinyl)ethyl chloride hydrochloride in the presence of potassium carbonate to produce the O-alkylated derivative, methyl 4-[2-(4-morpholinyl)ethoxy]benzoate which was further hydrolyzed to give 4-[2-(4-morpholinyl)ethoxy]benzoic acid. This acid was converted into its corresponding acid chloride by reacting with thionyl chloride which was further condensed with 3,5-bis(4-chlorobenzylidene)-4-piperidone in the presence of triethylamine to provide **2** as the free base. This free base was converted to its hydrochloride salt by treating with dry hydrogen chloride gas. The purity of **2** was established by ^{1}H NMR spectroscopy and elemental analysis.

Compound **2** was screened against the drug-sensitive D6 and the C235 drug-resistant strains of *Plasmodium falciparum* using the SYBR Green-I based fluorescence assay.[14] In brief, different concentrations of the compound were added to the parasites in a supplemental RPMI 1640 medium at an initial parasitemia of 0.3% and a haematocrit of 2% for the dose response determinations.

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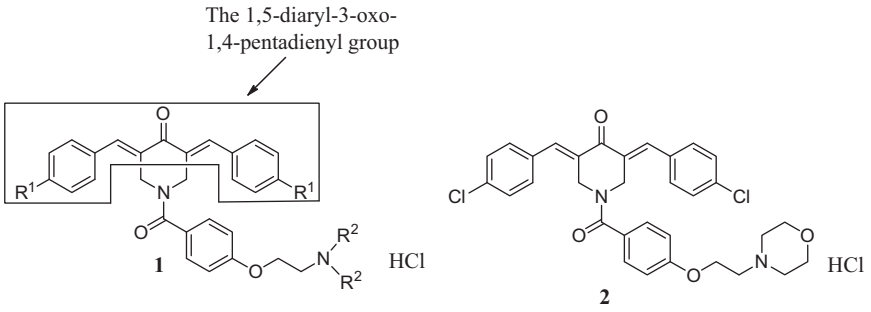

Figure 1. The structures of series **1** and compound **2**.

The cultures were incubated at 37 °C in a humidified atmosphere of carbon dioxide (5%), oxygen (5%) and nitrogen (90%) for 72 h. A lysis buffer containing SYBR Green-1 dye was added to each well. After incubation for 24 h in the dark at room temperature, the plates were examined for relative fluorescence. The antimalarial potencies (IC_{50} values) of **2** were generated with a GraphPad Prism (GraphPad Software Inc., San Diego, USA) using a nonlinear regression (sigmoidal dose-response/variable slope) equation. The IC_{50} value of **2** towards D6 parasites is 0.37 μg/mL (0.60 μM) and in this assay, the IC_{50} values for the established antimalarial drugs chloroquine and mefloquine are 6.7 ng/mL (0.02 μM) and 7.6 ng/mL (0.02 μM), respectively. The drug-resistant C235 strain of *P. falciparum* contains multiple copies of the pfmdr1 transporter gene and is resistant to many antimalarial drugs. The IC_{50} value of **2** to this protozoan is 1.21 μg/mL (1.97 μM) in contrast to the values of 78.5 ng/mL (0.25 μM) for chloroquine and 19.7 ng/mL (0.05 μM) for mefloquine. Hence the cytotoxic compound **2** displays promising growth-inhibiting properties to both drug-sensitive and drug-resistant strains of *P. falciparum*. The difference between the potencies of **2** towards D6 and C235 parasites is 3.3. This figure compares favorably to a potency difference of 12 for chloroquine and is similar to a value of 2.6 for mefloquine.

In addition, the ability of **2** to partition into erythrocytes, its effect on GSH concentrations in red blood cells and its permeability characteristics were evaluated. All procedures involving animals were conducted in accordance with the Canadian Council of Animal Care guidelines for the care and use of laboratory animals and were approved by the Animal Research Ethics Board of the University of Saskatchewan.

The reason for assessing **2** for its ability to concentrate in red blood cells is that one of the stages of the life cycle of the malarial parasite is located in the erythrocytes. After a mosquito bite, the sporozoites multiply in the liver forming merozoites which, following the rupture of the host cells, pass into the blood stream and invade red blood cells. Thus a positive tropism of a candidate antimalarial for erythrocytes is a significant advantage. For erythrocyte partition experiments, blood, which was collected by cardiac puncture from male Wistar rats under isoflurane anaesthesia, was placed in tubes containing ethylenediaminetetracetic acid. An aliquot of a stock solution of **2** in dimethylsulfoxide (2.5 mg/mL)

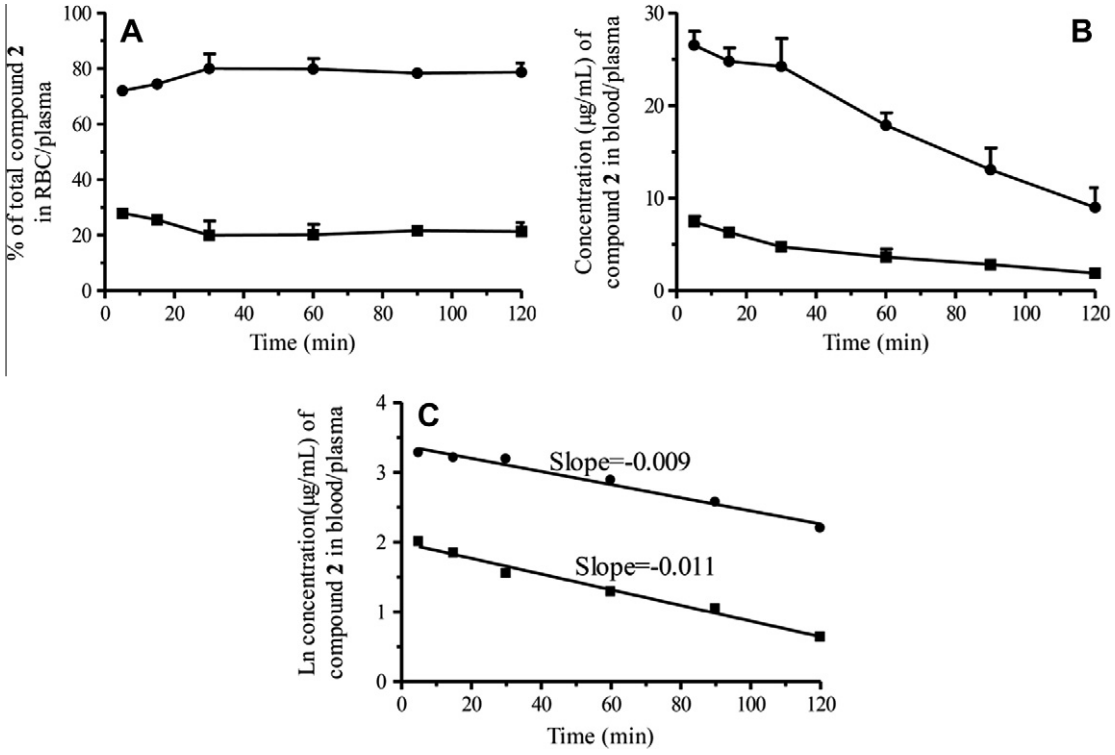

Figure 2. The concentrations of **2** in erythrocytes (●) and plasma (■) expressed as the percentage of total compound (A) and also in μg/mL in both linear (B) and logarithmic (C) relationships.