Toward a Novel Metal-Based Chemotherapy against Tropical Diseases. 3. Synthesis and Antimalarial Activity *in Vitro* and *in Vivo* of the New Gold−Chloroquine Complex $[Au(PPh_3)(CQ)]PF_6$

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Reaction of $AuPPh_3Cl$ with chloroquine (CQ) and KPF_6 leads to the new complex $[Au(PPh_3)(CQ)]PF_6$ (**1**) which was found to be considerably more active than CQ diphosphate and other previously reported metal−CQ complexes against two chloroquine-resistant strains of *Plasmodium falciparum in vitro* and also active against *Plasmodium berghei in vitro* and *in vivo*.

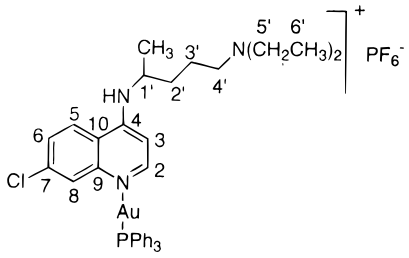
Malaria is by far the most serious tropical disease, currently affecting about 400 million people and threatening more than 1 billion people in the world.[1] Although metals have been used in medicine for centuries, the success of *cis*-$PtCl_2(NH_3)_2$ (cisplatin) as an anticancer drug[2,3] has stimulated a renewed interest in metal-based chemotherapies. There is little doubt that other new metal-containing drugs will be developed in a variety of therapeutic areas, including the treatment of parasitic diseases, as the field of inorganic medicinal chemistry continues to grow.[2b]

In previous papers of this series[4] we have described our strategy for the development of alternative metal-based drugs against tropical diseases. We have disclosed, for instance, that the complex $RuCl_2(CTZ)_2$ (CTZ = clotrimazole) displays good activity against *Trypanosoma cruzi*,[4a] while $[RuCl_2(CQ)]_2$ (CQ = chloroquine) is efficient against *Plasmodium falciparum*,[4b] the parasites responsible for Chagas disease and malaria, respectively.

Gold compounds have long been used for the treatment of various diseases,[6a] notable examples being aurothiomalate and aurothioglucose complexes for rheumatoid arthritis[5] and cyanide and thiosulfate derivatives for tuberculosis.[2b] The gold complex auranofin proved to be active also against P388 leukemia.[6a] In contrast, the potential of gold derivatives as antiparasitic agents has so far been very little explored.[2b] Continuing our efforts to develop improved antimalarial drugs, we now report that coordination of CQ to the $[Au(PPh_3)]^+$ fragment produces a new compound which is very active against *in vitro* cultures of chloroquine-resistant strains of *P. falciparum*, a particularly dangerous parasite for humans, and also against *Plasmodium berghei* (rodent malaria) *in vitro* and *in vivo*.

Chemistry

Synthesis of $[Au(PPh_3)(CQ)]PF_6$ (1). Reaction of $AuClPPh_3$[7] with CQ and KPF_6 in CH_3CN leads to the new complex $[Au(PPh_3)(CQ)]PF_6$ (**1**) which is isolated in good yields as air stable orange microcrystals. In the FAB-MS spectrum of **1**, the cation is registered as the parent peak in high intensity, associated with fragment ions of the expected pattern; the IR spectrum displays peaks associated with coordinated PPh_3 and CQ, plus a band characteristic of the PF_6 anion. All NMR



(**1**)

resonances could be assigned on the basis of 1D and 2D correlated COSY and HETCOR experiments. The largest ^1H chemical shift variation with respect to the free ligand is observed for H(8) ($\Delta\delta$ 0.26), located in the vicinity of N(1), while all other chloroquine protons shift by less than 0.19 ppm except NH which moves 0.45 ppm downfield; as discussed previously[4b] this pattern indicates that CQ binds to gold through the unsubstituted N(1) atom, a good donor site of this molecule. The formulation for **1** thus corresponds to a 14-electron configuration for Au(I), most probably in the usual linear coordination geometry,[8] which has been previously associated with biological activity.[2b,6] Since the biological tests were carried out using DMSO as the solvent, it is important to note that the NMR spectra of **1** in DMSO-d_6 remain unchanged for several days at 30 °C, showing no evidence of displacement of the CQ ligand by the solvent or any other sign of decomposition.

Results and Discussion

Preliminary experiments demonstrated that complex **1** caused a marked inhibition of the *in vitro* growth of the rodent malarial parasite *P. berghei*. The IC_{50} value of CQ base determined for $[Au(PPh_3)(CQ)]PF_6$ was 3×10^{-9} M, about *22 times lower* than the concentration of CQ required for chloroquine diphosphate CQDP (6.7×10^{-8} M). This promising activity of **1** against *P. berghei* encouraged us to test its potential antimalarial action on *P. falciparum*. The results collected in Table 1 show that **1** was also very effective against two chloroquine-resistant strains of *P. falciparum*. The IC_{50} values of CQ base for **1** were 5.1×10^{-9} and 2.3×10^{-8} M against the FcB1 and FcB2 strains, respectively; the corre-

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Table 1. Effect of CQDP and Complex **1** on the *in Vitro* Growth of Two Chloroquine-Resistant Strains of *P. falciparum*[a]

compound	IC_{50} (M)[a]	
	FcB1	FcB2
$[Au(PPh_3)(CQ)]PF_6$	5.1×10^{-9}	2.3×10^{-8}
CQDP	4.7×10^{-8}	1.1×10^{-7}

[a] IC_{50} = 50% inhibitory concentration of CQ base.

Table 2. Effect of CQDP and Complex **1** on *P. berghei* Parasitemia in BALB/c Mice[a]

treatment	% parasitemia (mean ± SD)
none	45 ± 7.2
CQDP	25 ± 3.1
$[Au(PPh_3)(CQ)]PF_6$	7 ± 1.3

[a] Average parasitemia of nontreated control and experimental mice treated with CQDP or complex **1** at 1 mg of CQ/kg of body weight daily from day 0 to day 3 are shown. Levels of parasitemia were determined on day 4 of infection; 7 mice/group.

sponding IC_{50} values of CQ base for CQDP were 4.7×10^{-8} M against the FcB1 and 1.1×10^{-7} M for the FcB2. Thus, the enhancement of the activity of CQ caused by coordination to Au, estimated from the ratios IC_{50}(CQDP)/IC_{50}(**1**), was 9.2 for FcB1 and 4.8 for FcB2. These results represent *a marked improvement* with respect to the activity of $[RuCl_2(CQ)]_2$ recently reported by us.[4b] Neither control cultures treated with medium containing 0.16% (v/v) DMSO in concentrations equivalent to the level of DMSO in the culture containing complex **1** nor the CQ-free complex $AuClPPh_3$ in DMSO caused any inhibition of parasite growth; this indicates that the observed inhibition was due to the presence of *both CQ and gold* in the medium.

In a further series of experiments the *in vivo* activity of complex **1** against *P. berghei* was evaluated. Inoculations into BALB/c mice were made by the intravenous injection of 100 μL of blood suspension (10^7 parasites) in sterile physiological saline. Parasitemia was monitored by microscopic examination of Giemsa-stained blood smears on glass slides. For each slide counts were made of 1000 erythrocytes, and the mean and standard deviation were calculated for 7 mice/group. Drug-treated mice received the standard[10a] 4 day treatment in which animals are inoculated on day 0, treated once a day from day 0 to day 3, and then examined for parasitemia levels on day 4. All compounds were administered by the intraperitoneal route. Corresponding placebo was administered to control mice. Preliminary testing of compound **1** for adverse effects on mouse well-being was carried out by monitoring reactions at the site of injection as well as 30 day survival in mice given three ip injections at the dose of 5.4 mg of **1**/kg. No apparent adverse reactions or apparent toxic responses were observed. Using the standard 4 day suppressive test,[10a] the ED_{50} value of CQ determined for CQDP was 1 mg/kg, a value which is in agreement with previous data from our laboratory.[4b,10c] In order to assess the *in vivo* activity of **1** against *P. berghei*, levels of parasitemia were estimated in mice receiving 1 ED_{50} of CQ (1 mg/kg) given as either CQDP or the equivalent concentration of the base in complex **1**. The results of this test are collected in Table 2. The level of parasitemia of mice treated with **1** was significantly lower ($p < 0.0001$) than that of animals treated with CQDP as determined by the Student's *t*-test. A concentration of CQ in **1** which was equivalent to 1 ED_{50} of the parental compound was sufficient to suppress parasitemia by 84% as compared to untreated controls, showing that complexation of Au to CQ *significantly increased* the *in vivo* susceptibility of *P. berghei* to CQ.

Conclusion

The new Au−CQ complex **1** synthesized by reaction of CQ base with an appropriate metal precursor displayed high *in vitro* activity against the blood stage of two chloroquine-resistant strains of *P. falciparum*, considerably superior to those of other metal−CQ complexes (Ru, Rh) previously evaluated. Compound **1** was also active *in vitro* and *in vivo* against *P. berghei* showing that the incorporation of the gold fragment produced a marked enhancement of the efficacy of chloroquine. We are currently attempting to modify the chemical structure of **1**, in order to achieve higher *in vivo* activities, and are also investigating the possible modes of action of this very promising compound.

Experimental Section

Chemistry. All manipulations were routinely carried out under N_2 using Schlenk techniques. Solvents were purified by standard procedures immediately prior to use. The extraction of chloroquine base was described before.[2b] All other commercial reagents were used without further purification. NMR spectra were recorded on a Bruker AM 300 spectrometer. The IR spectra were obtained with a Nicolet 5DCX FT instrument. Elemental analyses were performed by use of a EA 1108 FISONS instrument, and positive ion FAB mass spectra were obtained in matrices of methanol−nitrobenzyl alcohol (NBA) at the analytical services of the University of California Riverside mass spectrometry facility.

$[Au(PPh_3)(CQ)]PF_6 \cdot 2H_2O$ (1). A suspension of $AuClPPh_3$ (0.53 g, 1.07 mmol) in acetonitrile (40 mL) was refluxed under nitrogen until complete dissolution, then KPF_6 (0.33 g, 2.14 mmol) was added, and refluxing was continued for 30 min. Chloroquine (0.68 g, 2.14 mmol) was added, and the mixture was stirred and refluxed for 48 h, then cooled to room temperature, and filtered through Celite. The volume of the solvent was reduced under a nitrogen stream, and diethyl ether was added until the solution became turbid; on cooling to −5 °C overnight the orange product precipitated. It was filtered off, washed with water and diethyl ether, and dried under vacuum: yield 90%; FAB-MS (MeOH/NBA) $(M - PF_6)^+$ = 778, $(M^+ - CQ)$ = 459; IR ν(N−H) 3416, ν(C=C) 1613, ν(C=N) 1591, $\nu(PF_6)$ 843 cm^{-1}; ^1H NMR ($(CD_3)_2CO$) ppm 8.55 (d, J = 6.04 Hz, H2), 6.76 (d, J = 6.23 Hz, H3), 8.31 (d, J = 9.02 Hz, H5), 7.46 (dd, ^3J = 9.04 Hz, ^4J = 2.19 Hz, H6), 8.16 (d, J = 2.10 Hz, H8), 3.86 (br, H1′), 1.33 (d, J = 6.40 Hz, H1″), 1.65 (m, H2′, H3′), 2.56 (m, H4′, H5′), 0.98 (t, J = 7.16 Hz, H6′), PPh_3 7.67 (m, 15H); $^{13}C\{^1H\}$ NMR ppm 152.6 (C2), 99.9 (C3), 148.6 (C4), 124.4 (C5), 127.0 (C6), 135.8 (C7), 125.8 (C8), 151.6 (C9),118.2 (C10), 49.0 (C1′), 20.0 (C1″), 33.4 (C2′), 22.0 (C3′), 47.9 (C4′), 52.6 (C5′), 9.6 (C6′); PPh_3 135.9 C_{ortho}, 130.4 C_{meta}, 134.8.C_{para}; $^{31}P\{^1H\}$ NMR PPh_3 33.2 (s), PF_6^- 141.3 (hep). Anal. ($AuC_{36}H_{45}N_3ClO_2PF_6$) C, H, N.

Biology. Parasites. The FcB1 and FcB2 Colombian chloroquine-resistant strains of *P. falciparum* were obtained from S. Herrera (Universidad del Valle, Cali, Colombia). Parasites were cultured continuously[9a] on human red blood cells (O^+) in RPMI 1640 medium (GIBCO-BRL) supplemented with 25 mM HEPES (*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid), 32 mM $NaHCO_3$, 2 mM L-glutamine, 10 mM glucose (Sigma), and 10% (v/v) inactivated (56 °C/40 min) human plasma O^+. The latter had been collected in acid−citrate−dextrose anticoagulant. Stock cultures were maintained in 5 mL of 6% erythrocyte suspension in 25 mL tissue culture flasks (Corning). The strain of *P. berghei* used was obtained from Dr. F. Pifano, Venezuelan Institute of Tropical Medicine "Felix Pifano", in 1988. Parasites are kept frozen

in liquid nitrogen as a 1:2 dilution of infected blood (10−15% infection) in 28% glycerol−3% sorbitol.

***In Vitro* Tests.** Antimalarial activity of compound **1** was first assessed on short-term *in vitro* cultures of *P. berghei* and then further evaluated on *P. falciparum*. The working solution of **1** was 18 mM in RPMI medium containing 0.16% (v/v) DMSO (Sigma). CQ diphosphate (Sigma) was diluted in RPMI 1640 medium without serum/plasma to obtain a working solution of 19.4 μM. Drug testing was performed in triplicate in 96-well, flat-bottomed plates (Linbro) with cultures containing mostly ring stages. Short-term cultures of *P. berghei* were obtained according to the protocol described by Kamiyama and Matsubara.[9b]

In brief, infected blood was obtained from mice harboring low parasitemias (3−5%) for which predominant blood stages were rings and young trophozoites (one nucleus). Blood was washed with RPMI medium supplemented with HEPES (25 mM), L-glutamine (2 mM), sodium bicarbonate (18 mM), reduced glutathione (3.25 μM), and 5% (v/v) normal rat serum which had been previously inactivated at 56 °C/30 min. The same batch of rat serum gathered from animals 4−6 weeks old was used throughout the experiments. Suspensions of normal and infected erythrocytes in complete culture medium were mixed to obtain a working cell suspension containing 2 $\times$ 10^7 parasitized cells/mL and 1.8 $\times$ 10^8 uninfected erythrocytes/mL. Uninfected erythrocytes (2 $\times$ 10^8/mL) served as controls. One hundred microliters of the cell suspension and 25 μL of the tested compound or medium alone (with or without 0.16% DMSO) were mixed in the appropriate well followed by addition of 25 μL of the [^{3}H]hypoxanthine solution (Amersham International) (0.5 μCi) in culture medium. After a 24 h incubation period in an atmosphere of 5% CO_2, 10% O_2, and 85% N_2, the cells were collected on glass-fiber disks (934 AH, Whatman) using a cell harvester (MASH II, Microbiological Associates) and counted in a liquid scintillation counter (LKB 1217 Rackbeta). Dose−response curves for each compound were generated and the IC_{50} values calculated from the corresponding regression lines.

Microcultures of *P. falciparum* were prepared after Desjardins et al.[9c] Samples of stock cultures of the FcB1 and FcB2 strains of *P. falciparum* were diluted in culture medium containing sufficient noninfected human O^+ erythrocytes to give a final hematocrit of 1.5% and parasitemia of 0.25−0.5%. Two hundred microliters of the infected erythrocytes was mixed in each well with 25 μL of the tested compound or medium alone (with or without 0.16% (v/v) DMSO). Microplates were incubated at 37 °C using the candle jar method.[9a] After a 24 h incubation period, 25 μL of the [^{3}H]hypoxanthine solution (0.5 μCi) in culture medium was added to each well. The plates were further incubated at 37 °C during 18 h, cells were harvested and counted, and IC_{50} values were calculated as indicated above.

***In Vivo* Test: Animals and Infection**. Experimental mice were female BALB/c mice, 2−3 months old, from the animal breeding unit of IVIC. Mice were kept in plastic cages and received standard food and water *ad libitum*. To initiate infections, the *P. berghei* parasites were passaged once or twice in BALB/c mice before use in each experiment. Experimental mice received 10^7 parasitized erythrocytes given intravenously (iv), and parasitemias were monitored daily by counting the number of infected erythrocytes/1000 erythrocytes on tail blood smears stained with Giemsa.

Administration of Drug. CQDP was dissolved in 0.9% (w/v) NaCl to give the dose required in 0.1 mL/10 g of mouse and injected intraperitoneally (ip) into mice daily for 3 consecutive days from day 0 of infection.[10] Complex **1** was dissolved in DMSO, diluted 10-fold with Intralipid (200 g of fractionated soybean, 12 g of fractionated phospholipids, 22 g of glycerol USP, 1000 mL of water qsp; Kavi Vitrum, Stockholm, Sweden), and then injected ip in mice daily from day 0 to day 3 of infection. Drug treatment started 2 h after inoculation. Levels of parasitemia were determined on day 4. The results are expressed as the percentage of infected cells.[10b] Previous assays indicated that four daily ip injections of DMSO diluted 10-fold in Intralipid did not affect the course of infection. The levels of parasitemias of control and drug-treated mice were compared by the Student's *t*-test. To obtain values for 50% effective levels of the parental compound (ED_{50}), the percentage of erythrocytes containing parasites in the treated group was compared to that of controls and related to the log dose of the drug. The ED_{50} value was calculated from the corresponding regression line.

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