Toward a Novel Metal-Based Chemotherapy against Tropical Diseases. 7. Synthesis and in Vitro Antimalarial Activity of New Gold−Chloroquine Complexes

Maribel Navarro,*,§ Flor Vásquez,§ Roberto A. Sánchez-Delgado,*,§,# Hilda Pérez,‡ Véronique Sinou,+ and Joseph Schrével†

Transition Metal Chemistry and Inmunoparasitology Laboratories, Instituto Venezolano de Investigaciones Cientificas (IVIC), Apartado 28127, Caracas 1020-A, Venezuela, Muséum National d'Histoire Naturelle, Laboratoire de Biologie Parasitaire, USM 0504 Biologie Fonctionnelle des Protozoaires, 75231 Paris Cedex 05, France, and Institut de Médecine Tropicale du Service de Santé des Armées, Le Pharo BP 46, 13998 Marseille Armées, France

Received March 15, 2004

A number of new Au(I) and Au(III) complexes of chloroquine (CQ) have been prepared, characterized, and evaluated in vitro against several strains of *Plasmodium falciparum*. [(CQ)-Au(PPh_3)][NO_3] (**2**) was synthesized by reaction of Au$Cl(PPh_3)$ with $AgNO_3$ followed by treatment with CQ. Similar reactions of Au$Cl(PR_3)$ (R = Me, Et) with KPF_6 and CQ yielded [(CQ)Au(PMe_3)][PF_6] (**3**), and [(CQ)Au(PEt_3)][PF_6] (**4**), respectively. $KAuCl_4$ reacted with CQ to produce the Au(III) complex [$(CQ)_2Au(Cl)_2$]Cl (**5**), which in turn formed [(CQ)Au(Cl)(SR)-(Et_2O)]Cl (**6**) by reaction with 1-thio-β-D-glucose-2,3,4,6-tetraacetate (SRH). The new compounds were characterized by a combination of elemental analysis, fast atom bombardment mass spectrometry (FAB-MS), and NMR spectroscopy. All the complexes display in vitro activity against CQ-sensitive and CQ-resistant strains of *Plasmodium falciparum*. The highest activity for this series was obtained for complex **4**, which is 5 times more active than chloroquine diphosphate (CQDP) against the CQ-resistant strain FcB1. On preincubation of noninfected red blood cells with complexes **1**, **5**, and **6**, protection against subsequent infection was observed in some cases. No clear structure−activity correlations could be established for this series of compounds.

The rapid spread of drug-resistant malaria in southeast Asia and the threat of resistant strains of *Plasmodium falciparum* spreading to Africa, India, and South and Central America has stimulated the search for new drugs to treat the more than 400 million people infected with this parasite.[1] There is an urgent need for antimalarials with novel structures, modes of action, or both to deal with the development of resistance to the drugs in current use. As previously demonstrated by some of us,[2] attaching organic drugs to metal-containing fragments can enhance their activity, for example, chloroquine for malaria and clotrimazole for Chagas disease. Although metals have been used in medicine for centuries, the success of *cis*-$PtCl_2(NH_3)_2$ (cisplatin) and related compounds as anticancer drugs[3,4] has stimulated a renewed interest in metal-based chemotherapies. There is little doubt that new metal-containing drugs will be developed for a variety of therapeutic applications as the field of inorganic medicinal chemistry continues to grow.[3b]

Gold compounds have long been used for the treatment of various diseases,[5] notably aurothiomalate and aurothioglucose complexes for rheumatoid arthritis.[6]

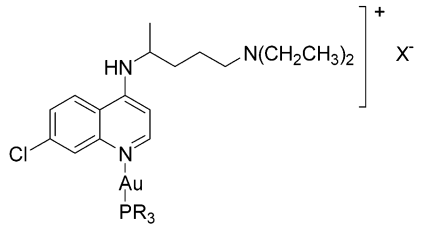


Figure 1. Proposed structure for complexes [(CQ)Au(PR_3)]-[X] (**1**, R = Ph, X = PF_6; **2**, R = Ph, X = NO_3; **3**, R = Me, X = PF_6; **4**, R = Et, X = PF_6).

The drug auranofin is also active against P388 leukemia.[7] In contrast, the potential of gold derivatives as antiparasitic agents has not yet been fully investigated.[3b] In a previous publication we disclosed the synthesis of [(CQ)Au(PPh_3)][PF_6] (**1**) (see Figure 1), as well as data on its in vitro activity against two CQ-resistant strains of *Plasmodium falciparum* and its in vitro and in vivo activity against *Plasmodium berghei*.[2c] Continuing with our efforts to develop improved antimalarial drugs, we herein report a series of new CQ−Au derivatives, as well as their in vitro activity against several strains of *P. falciparum*.

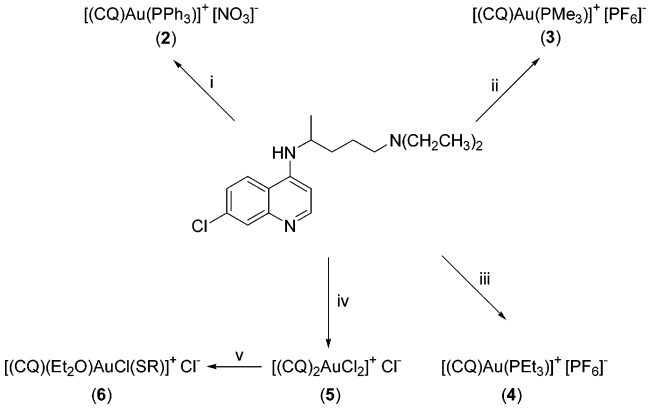
Chemistry

Synthesis and Characterization of New Gold Complexes. We have previously reported that Au$Cl(PPh_3)$ reacts with 1 equiv of KPF_6 and CQ in CH_3CN to yield the complex [(CQ)Au(PPh_3)][PF_6] (**1**).[2c] Using similar procedures, we have now achieved the synthesis

* To whom correspondence should be addressed. For R.A.S.-D.: phone, +58 212 504 1741; fax, +58 212 514 1350; e-mail, rsanchez@ivic.ve. For M.N.: phone, +58 212 504 1642; fax, +58 212 504 1350; e-mail, mnavarro@ivic.ve.
# John Simon Guggenheim Fellow, 1998−1999.
§ Transition Metal Chemistry Laboratory, IVIC.
‡ Immunoparasitology Laboratory, IVIC.
+ Institut de Médecine Tropicale.
† Muséum National d'Histoire Naturelle.

10.1021/jm049792o CCC: $27.50
Published on Web 09/04/2004

Scheme 1. Synthetic Procedures[a]



[a] (i) $AuClPPh_3/AgNO_3$/CQ 1:1:2 in acetonitrile, rt; (ii) $AuClPMe_3/KPF_6$/CQ 1:2:2 in acetonitrile under reflux; (iii) $AuClPEt_3/KPF_6$/CQ 1:2:2 in acetonitrile under reflux; (iv) $KAuCl_4$/CQ 1:2 in acetone, rt; (v) SRH (1-thio-β-D-glucose-2,3,4,6-tetraacetate) 1:1 in acetone, −10 °C.

of a number of related Au−CQ derivatives, as summarized in Scheme 1. This has allowed us to probe the effect of varying the coordination sphere around the metal ion on the biological properties of the complexes.

A first point of interest was to investigate whether the nature of the counteranion in complexes such as **1** has any biological implications. The reaction of $AuCl(PPh_3)$ with 1 equiv of $AgNO_3$ in CH_3CN followed by treatment with CQ was the most efficient way to prepare $[(CQ)Au(PPh_3)][NO_3]$ (**2**), which was isolated in good yields as a yellow solid. While our work was in progress, the same compound was independently synthesized by the group of Moss by a slightly different procedure.[13] The use of phosphine ligands (PR_3) is important for our purposes since it is well-known that variations in the nature of the R group can induce notable changes in the electronic and steric properties of the ligand. This, in turn, can produce important modifications of the chemical and biological properties of the resulting metal complexes. The systematic variation of the phosphine ligand was achieved by reacting preformed $AuCl(PMe_3)$ or $AuCl(PEt_3)$ with an excess of KPF_6 in acetonitrile, followed by addition of CQ;[2g] this led to the corresponding derivatives $[(CQ)Au(PMe_3)][PF_6]$ (**3**) and $[(CQ)Au(PEt_3)][PF_6]$ (**4**). A related phosphine-free Au(III)−CQ complex $[(CQ)_2Au(Cl)_2]Cl$ (**5**) could be obtained from the reaction of $KAuCl_4$ with 2 equiv of CQ in acetone. A final consideration regarding the series of reactions described in Scheme 1 is related to the well-known use of Au(III) complexes in the treatment of arthritis; a particularly useful compound for such applications is auranofin $[(PEt_3)Au(SR)]$ (where ^-SR = 1-thio-β-D-tetraacetateglucose). There is some evidence that chloroquine diphosphate (CQDP) also has important antiarthritic properties[8] and thus it seemed interesting to combine CQ and ^-SR on the same metal complex to evaluate the antimalarial activity of a compound containing both functionalities; this was achieved by reaction of complex **5** with 1-thio-β-D-glucose-2,3,4,6-tetraacetate in acetone at −10 °C, which led to the complex $[(CQ)\ Au(Cl)(SR)(Et_2O)]Cl$ (**6**). It is also of interest to contrast the biological properties of complexes **2**−**4**, containing Au(I) ions, with those of **5** and **6**, which are Au(III) derivatives.

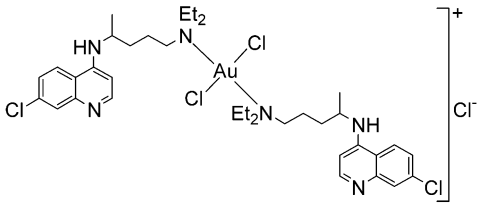


Figure 2. Proposed structure of $[(CQ)_2Au(Cl)_2]Cl$ (**5**).

All the new compounds were characterized by a combination of elemental analysis, fast atom bombardment mass spectrometry (FAB-MS), and IR and NMR spectroscopy, as described in the Experimental Section. The FAB-MS spectra displayed parent peaks of high intensity, corresponding to the cations of the complexes. The IR spectra displayed peaks clearly associated with the presence of the coordinated ligands and of the counteranions where present. All ^1H NMR resonances could be unequivocally assigned on the basis of 1D- and 2D-correlated correlation spectroscopy (COSY) and HETCOR experiments (for complete NMR data as well as the atom numbering used, see Experimental Section). Selected data of relevance for the discussion that follows are contained in Table S1 (Supporting Information). We have used the ^1H chemical shift variation of each signal with respect to those of the free ligand ($\Delta\delta$) as a parameter to deduce the mode of bonding of CQ to the metal. It has been previously shown by us[2] and by others[9] that the largest variations are always observed for the protons located in the vicinity of the N-atom attached to the metal. According to the data available, the formulation for **1**−**4**, represented in Figure 1, corresponds to a 14-electron Au(I) configuration, most probably in the usual linear coordination geometry[10] that has been previously associated with biological activity.[2c,5−6] In all these complexes, the largest ($\Delta\delta$) values are observed for H(8) (see Table S1, Supporting Information), indicating that CQ is bound to the metal through the N(1) atom of the quinoline moiety, a good donor site of this molecule.

Similarly, we deduce that the formulation of **5** corresponds to a 16-electron Au(III) configuration with a square planar coordination containing mutually trans pairs of Cl and CQ ligands. Au(III) is isoelectronic with Pt(II) and the complexes of both metals in such oxidation states normally adopt a square planar geometry. This similarity has been previously associated with a biological activity of Au(III) in parallel to cisplatin and its homologues.[11] Square planar Au(III) derivatives have also been reported to display activity against a range of microbial strains and several human tumor lines.[12] The NMR data in this case (Table S1) are consistent with CQ binding to the metal through the N(3) atom of the terminal diethylamino group of the alkyl chain, as depicted in Figure 2,[10] since the largest ($\Delta\delta$) values were observed for H(4′) and H(5′).

In accordance with the microanalytical data, the molecular ion displayed in the FAB-MS and the NMR spectra (see Experimental Section), compound **6**, was found to contain the gold atom bonded to one CQ molecule through the N(3) atom and to one thioglucose ligand; a third coordination site is occupied by a chloride and the square planar arrangement is completed by a solvent (diethyl ether) molecule (see Figure 3). The

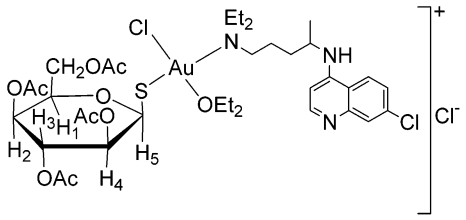


Figure 3. Proposed structure of [(CQ)(Et_2O)Au(Cl)(SR)][Cl] (**6**).

Table 1. Effects of CQDP and Au−CQ Complexes on the in Vitro Growth of Several strains of *P. falciparum*

	IC_{50}[a] (rel activ)[b]			
	FcB1	W2	K1	F32
CQDP[c]	50 (1.00)	70 (1.00)	91 (1.00)	10 (1.00)
1	40 (1.25)	70 (1.00)	99 (0.92)	18 (0.55)
2	40 (1.25)	40 (1.75)	58 (1.57)	10 (1.00)
3	40 (1.25)	50 (1.40)	93 (0.98)	17 (0.59)
4	10 (5.00)	50 (1.40)	99 (0.92)	13 (0.77)
5			43 (2.11)	13 (0.77)
6			54 (1.68)	18 (0.55)

[a] IC_{50} = 50% inhibitory concentration of CQ base (in nM). Each concentration was estimated from independent experiments in triplicate. [b] Rel activ = IC_{50}(CQDP)/IC_{50}(complex). [c] Based on the IC_{50} values for CQDP, the FcB1, W2, and K1 strains are considered of medium CQ resistance, while F32 is CQ sensitive.

charge of this cation is compensated by a noncoordinated chloride ion. Presumably, when complex **5** reacts with thioglucose one of the chlorides is replaced by the RS^- anion, and to avoid strong steric repulsions between three voluminous ligands, one CQ is lost generating a flexible electron-deficient three-coordinate species in solution. Such a species will adopt the less sterically hindered mutually trans disposition of the two bulky ligands CQ and RS^-. Eventually, the more stable four-coordinated 16-electron configuration is attained by binding a labile solvent molecule as the fourth ligand, which is preferentially accommodated in the position trans to the chloride. This type of isomerization of metal complexes promoted by steric repulsions is very common in solution.[10]

Since all the biological tests were carried out using DMSO as the solvent, it was important to establish the stability of such solutions. The NMR spectra of all the complexes in DMSO-d_6 remained unchanged for several days at 30 °C, showing no evidence of displacement of the CQ ligand by the solvent nor any other sign of decomposition.

Results and Discussion

All the new compounds described in the previous section were evaluated in vitro against several strains of *Plasmodium falciparum*: F32 from Tanzania, FcB1 from Colombia, K1 and W1 from southeast Asia. As can be observed in the data collected in Table 1, all the metal derivatives described herein are active against CQ-sensitive (F32), as well as CQ-resistant (FcB1, K1 and W1), strains.

The activities displayed by these compounds are similar or superior to that of CQDP. It must be noted that in a previous publication by some of us,[2c] compound **1** was reported to be about 10 times more active than CQDP in in vitro tests against the FcB1 strain of *P. falciparum*. This complex also displayed considerable in vitro and in vivo activity against *P. berghei* in several experiments.[2c] However, the new tests reported here for the FcB1 strain, which have been conducted with freshly prepared samples of complex **1**, show it to be only 1.25 times higher than that of CQDP. In a similar way, complex **2**, synthesized independently by the group of Moss in South Africa was found to be about 6 times more active than CQDP against the K1 strain in their experiments,[13] whereas our results indicate it to be 1.6 times more active than CQDP for the same strain. The reasons for these discrepancies are unknown, but it is clear that the degree of drug resistance varies from one laboratory to another. Further work is in progress to try to clarify this point.

Table 2. Effect of Preincubation of Red Blood Cells (RBCs) in 100 μM Chloroquine (CQDP) or Au−CQ Complexes during Three Hours at 37 °C on the in Vitro Reinvasion of the K1 Strain of *P. falciparum*[a]

	parasitemia (%)
control	5.60
CQDP	5.67
1	0
5	5.96
6	3.75

[a] After being washed, the treated RBCs were exposed to normal schizont-infected RBCs, and the reinvasion was estimated 6 hours later.

The limited number of compounds available does not allow structure−activity relations (SAR) to be extracted from our data. Nevertheless, some interesting experimental facts deserve further mention. Concerning the Au(I) complexes, it is noted that on varying the counteranion from PF_6^- in **1** to NO_3^- in **2**, the activity remains essentially unchanged for FcB1, increases for W2 and K1, and decreases for F32. Also, varying the phosphine substituents from Ph in **1** to Me in **3** or Et in **4** did not cause a major effect in general. However, a notable exception is the PEt_3 derivative **4**, which, in the case of the FcB1 strain, was 5 times more active than CQDP and 4 times more active than **1**.

Complexes **5** and **6**, which differ from the other four compounds in that they have a higher-valent gold atom (+3), also proved to be more active than CQDP for both the resistant K1 and the sensitive F32 strains. Obviously, a more extensive series of related compounds is necessary to reach a clear activity trend as a function of the molecular structure or of the oxidation state of the metal.

In a further series of experiments conducted on a synchronized K1 strain, noninfected red blood cells (RBCs) were preincubated for 3 h at 37 °C with 100 μM gold complexes **1**, **5**, or **6**. After appropriate washing in RPMI culture medium, they were placed in contact for 6 h with RBCs parasitized with 44−48 h old schizonts, and the resulting parasitemia was subsequently determined. The results of these experiments are summarized in Table 2.

It was observed that CQDP did not offer any protection under these conditions, nor did complex **5**. However, RBCs treated with complex **1** did not show any parasitemia, although lysis of the red cells was evident. In the case of complex **6**, a lower parasitemia was observed in comparison with the control or with CQDP-treated cells (3.75% vs. 5.60% in the control). Microscopic observations revealed that the parasites did not develop

as rings and appeared to be dead. If the compounds that induced protection are compared, it is noted that complex **1** is a cationic Au(I) species containing CQ and PPh_3 ligands, whereas **6** (of lower activity) is also cationic and contains Au(III), CQ, and RS^- ligands.

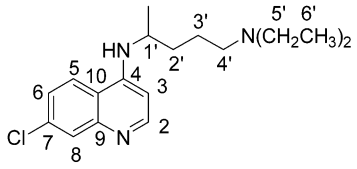
On the other hand, complex **5**, which caused no effect in this experiment, is also a cationic Au(III) complex, but it contains only CQ and Cl as ligands. Thus it seems that the presence of CQ together with a second stabilizing ligand different from chloride is more advantageous, whereas the oxidation state of the metal would not play an important role. PPh_3 is a neutral labile ligand that can be displaced easily from the cation in $[Au(CQ)(PPh_3)][PF_6]$ (**1**) at the level of the membrane of the RBC, liberating or exchanging an electron-deficient "$-[Au(CQ)]^+$" fragment capable of binding to electron-rich negatively charged membrane sites to form species of the type "Au(CQ)(-membrane)". The thioglucose moiety present in complex **6**, in turn, is anionic in nature and probably less labile but it is easily hydrolyzable by interaction with, for example, an acidic membrane site like −SH, leading to a membrane-bound species containing a similar motif "Au(CQ)(S−membrane)". The lower action observed in this case could be related to the neutral character of the complex, less favorable to be attracted to negatively charged sites. It is also possible that the role of the phosphine or thioglucose ligands is the stabilization of the "Au−CQ core" in its transit toward the appropriate membrane sites. Complex **5**, containing only CQ and chloride ligands is more readily hydrolyzed, and thus it may not be stable enough to reach the membrane in an active form. It is curious, however, that **5** is the most active complex against the K1 strain in the direct experiment (Table 1), followed by **6**, while **1** is actually inactive. Therefore, no relation is apparent between the activities observed in these two types of experiments, again pointing to the need to study a larger number of related compounds.

Conclusion

We have prepared and characterized a series of new Au(I) and Au(III) complexes containing CQ in combination with other ligands, which display activity against CQ-sensitive and CQ-resistant strains of *Plasmodium falciparum*. The highest activity for this series was obtained for $[(CQ)Au(PEt_3)][PF_6]$, which is 5 times more active than CQDP against the CQ-resistant strain FcB1. On preincubation of noninfected RBCs with the complexes, protection against subsequent infection was observed in some cases. Although this work confirms the concept previously proposed[2] that modification of an organic drug by introduction of a metal-containing fragment may increase its antimalarial activity, no clear structure−activity correlations could be established for this series of compounds.

Experimental Section

Chemistry. All manipulations were routinely carried out under N_2 using common Schlenck techniques.[14] Solvents were purified by standard procedures immediately prior to use. The extraction of chloroquine base from the diphosphate salt was performed as described before.[2b] All other commercial reagents were used without further purification. NMR spectra were obtained in DMSO-d_6 solution using a Bruker AVANCE 500 instrument operating at 500.13 MHz for ^1H and 202.45 MHz for ^{31}P. ^1H NMR shifts are recorded relative to the residual ^1H resonance in the deuterated solvent (2.49 ppm), and ^{31}P NMR shifts are relative to an external H_3PO_4 reference; the atom numbering for chloroquine is shown below.



IR spectra were obtained with a Nicolet 5DCX FT instrument. Elemental analyses were performed on a FISONS 1108 instrument, and positive ion FAB mass spectra were obtained in matrixes of methanol-nitrobenzyl alcohol (NBA) at the analytical services of the University of California Riverside mass spectrometry facility. HPLC analyses were carried out in a Hewlet Packard 1100 instrument fitted with a C18 column and operating in isocratic condition using as solvent mixture 50% ammonium acetate buffer (pH = 4.9), 30% methanol, 20% acetronitrile with a flow rate of 1 mL/min and detector lamp DAD.

$[(CQ)Au(PPh_3)][NO_3]$ (2). A suspension of $AuCl(PPh_3)$ (100 mg; 0.20 mmol) in CH_3CN (10 mL) was refluxed until complete dissolution was achieved; then $AgNO_3$ (34 mg; 0.20 mmol) was added, and a white precipitate was observed. After cooling to room temperature, the solution was filtered to remove the AgCl formed, and then chloroquine (129 mg; 0.40 mmol) was added. The mixture was stirred at room temperature for 30 min, the resulting yellow solution was filtered through Celite, the filter was washed with two portions of CH_3CN (1 mL each), and then the volume of the solvent was reduced until a yellow oil formed. The two phases were separated, and the oily residue was washed with water and diethyl ether and dried under vaccum to obtain a yellow solid. Yield 88%. M.p. 90 °C. FAB-MS (MeOH/NBA) $(M + H - NO_3)^+ = 778$, (CQ) = 320. IR (cm^{-1}): ν(N−H) 3250, ν(C═C) 1590, ν(C═N) 1600, $\nu(NO_3^-)$ 1385. ^1H NMR $(CD_3)_2SO$ δ (ppm): 8.54 (d, J = 6.19 Hz, 1H); 8.49 (d, J = 9.11 Hz, 1H); 8.15 (d, J = 2.00 Hz, 1H); 7.65−7.69 (m, 15H, PPh_3); 7.56 (dd, J^1 = 9.02 Hz, J^2 = 2.06 Hz, 1H); 6.71 (d, J = 6.41 Hz, 1H); 3.85−3.86 (m, 1H); 2.39−2.50 (m, 6H); 1.69−1.72 (m, 2H); 1.47−1.58 (m, 2H); 1.25 (d, J = 6.31 Hz, 3H); 0.91 (t, J = 6.96, 6H). $^{31}P\{^1H\}$NMR $(CD_3)_2SO$ δ (ppm): 31.04 (s, PPh_3). Anal. Calcd. ($C_{36}H_{41}N_4ClPO_3Au$) C, H, N.

$[(CQ)Au(PMe_3)][PF_6]$ (3). A suspension of $AuCl(PMe_3)$ (100 mg; 0.32 mmol) in CH_3CN (5 mL) was refluxed until complete dissolution was achieved; KPF_6 (119 mg; 0.64 mmol) was then added and refluxing was continued for 2 h. Chloroquine (207 mg; 0.64 mmol) was added, and the mixture was stirred under reflux for a further 48 h. Then the solution was cooled to room temperature and filtered throught Celite. The filter was washed with two portions of CH_3CN (1 mL each), the solvent was evaporated under a nitrogen stream to ca. 50% of its volume, and then diethyl ether was added until the solution became turbid; when the solution was cooled to −5 °C overnight, a yellow oil appeared. The solvent was decanted, and the remaining oil was washed with water and diethyl ether and dried under vaccum to obtain a yellow solid. Yield 92%. M.p. 132 °C. FAB-MS (MeOH/NBA) $(M + H - PF_6)^+ = 592$, (CQ) = 320. Purity by HPLC: 98%. IR (cm^{-1}): ν(N−H) 3400, ν(C═C) 1583, ν(C═N) 1617, $\nu(PF_6^-)$ 835. ^1H NMR $(CD_3)_2SO$ δ (ppm): 8.53 (d, J = 8.88 Hz, 1H); 8.42 (d, J = 6.19 Hz, 1H); 8.22 (s, 1H); 7.82 (br, 1H); 7.63 (d, J = 8.67 Hz, 1H); 6.76 (d, J = 5.95 Hz, 1H); 3.89 (br, 1H); 2.47 (br, 6H); 1.74−1.76 (m, 9H, $-CH_3$, PMe_3); 1.55−1.60 (m, 2H); 1.50 (br, 2H); 1.27 (d, J = 6.19 Hz, 3H); 0.93 (br, 6H). $^{31}P\{^1H\}$NMR $(CD_3)_2SO$ δ (ppm): 8.94 (s, PMe_3); −143.0 (hept, J = 708 Hz, PF_6^-).

$[(CQ)Au(PEt_3)][PF_6]$ (4). This compound was prepared by an analogous procedure to the one described for **3**, using $AuCl(PEt_3)$ (100 mg; 0.29 mmol) in CH_3CN (5 mL), KPF_6 (105 mg; 0.58 mmol), and chloroquine (182 mg; 0.58 mmol). Yield 68%. M.p. 118 °C. FAB-MS (MeOH/NBA) $(M + H - PF_6)^+ = 634$, (CQ) = 320. Purity by HPLC: 97%. IR (cm^{-1}): ν(N−H) 3420,

ν(C═C) 1585, ν(C═N) 1610, $\nu(PF_6^-)$ 838. ^{1}HNMR $(CD_3)_2SO$ δ (ppm): 8.60 (d, J = 9.08 Hz, 1H); 8.49 (d, J = 6.52 Hz, 1H); 8.35 (s, 1H); 8.09 (br, 1H); 7.70 (d, J = 9.02 Hz, 1H); 6.84 (d, J = 6.71 Hz, 1H); 3.96 (br, 1H); 2.51−2.50 (m, 6H); 2.03−2.04 (m, 6H, $-CH_2$, PEt_3); 1.71−1.74 (m, 2H); 1.60−1.62 (m, 2H); 1.28 (d, J = 6.30 Hz, 3H); 1.17−1.19 (m, 6H); 1.01 (br, 9H, $-CH_3$, PEt_3). $^{31}P\{^1H\}$NMR $(CD_3)_2SO$ δ (ppm): 32.63 (s, PEt_3); −143.0 (hept, J = 708 Hz, PF_6^-).

$[(CQ)_2Au(Cl)_2]Cl\cdot 2H_2O$ (5). To a solution of $KAuCl_4$ (300 mg, 0.79 mmol) in acetone (5 mL), chloroquine (508 mg, 1.58 mmol) in acetone (10 mL) was slowly added; the mixture was stirred for 2 h during which the color of the solution changed from orange to yellow. After 3 h, a white solid precipitated. The solution was filtered through Celite, the filter was washed with two portions of acetone (0.5 mL each), the solvent was evaporated to ca. 50% of its volume under a nitrogen stream, and diethyl ether was added until the solution became turbid; when the solution was cooled to −5 °C overnight, a yellow oil appeared. The two phases were separated, and the oily fraction was washed with water and diethyl ether and dried under vaccum to obtain a yellow solid. Yield 82%. M.p. 94 °C (dec). FAB-MS (MeOH/NBA) $[M - 3Cl^-]^+$ = 835, (CQ) = 320. IR (cm^{-1}): ν(N−H) 3278, ν(C═C) 1593, ν(C═N) 1614. ^{1}HNMR $(CD_3)_2SO$ δ (ppm): 8.42 (d, J = 5.49 Hz, 1H); 8.40 (d, J = 9.33 Hz, 1H); 7.79 (d, J = 2.02 Hz, 1H); 7.47 (dd, J^1 = 8.94 Hz, J^2 = 2.05, 1H); 7.09 (d, J = 7.78 Hz, 1H); 6.56 (d, J = 5.56 Hz, 1H); 3.80 (br, 1H); 2.90−2.97 (m, 6H); 1.72 (br, 4H); 1.60 (br, 2H); 1.26 (d, 3H); 1.13 (t, 6H). Anal. Calcd. ($C_{36}H_{56}Cl_5N_6Au\cdot 2H_2O$) C, H, N.

$[(CQ)(Et_2O)Au(Cl)(SR)]Cl$ (6) ($^-$SR = 1-thio-β-D-tetraacetateglucose). To a solution of $[(CQ)_2Au(Cl)_2]Cl$ (400 mg, 0.72 mmol) in acetone (15 mL) cooled to −10 °C, 1-thio-β-D-glucose tetraacetate (SRH) (264 mg, 0.72 mmol) in acetone (10 mL) was added. The mixture was stirred and maintained at −10 °C for 1 h, during which the pH changed from neutral to 3. The yellow solution was filtered throught Celite, the filter was washed with two portions of acetone (0.5 mL each), the solvent was evaporated under a nitrogen stream to ca. 50% of its volume, and then diethyl ether was added until the solution became turbid; when the solution was cooled to −5 °C overnight, a yellow oil appeared. The solvent was decanted off, and the remaining oil was washed with water and diethyl ether and dried under vacuum to obtain a yellow solid. Yield 84%. M.p. 86 °C. FAB-MS (MeOH/NBA) $(M + H - Cl)^+$ = 989, (CQ) = 320. IR (cm^{-1}): ν(N−H) 3416, ν(C═O) 1751, ν(C═C) 1614, ν(C═N) 1623. ^{1}HNMR $(CD_3)_2SO$ δ (ppm): 8.76 (d, J = 9.08 Hz, 1H); 8.51 (d, J = 6.65 Hz, 1H); 7.99 (br, 1H); 7.67 (dd, J^1 = 9.05 Hz, J^2 = 1.90 Hz, 1H); 6.84 (d, J = 6.72 Hz, 1H); 5.08 (m, 1H); 4.85−4.92 (m, 2H); 4.61−4.63 (m, 1H); 4.11 (br, 1H); 4.03 (br, 1H); 3.97 (br, 1H); 3.84 (br, 1H); 3.02−3.08 (m, 6H); 1.90−2.02 (m, 12H); 1.74 (br, 4H); 1.30 (d, J = 6.33 Hz, 3H); 1.19 (t, 6H). Anal. Calcd. ($C_{36}H_{56}N_3Cl_3O_{10}SAu$) C, H, N.

Biological Experiments

Culture and Synchronization of *Plasmodium falciparum*. The following strains of *Plasmodium falciparum* were used in this work: F32 from Tanzania (CQ-sensitive), FcB1 from Colombia, and K1 and W1 from southeast Asia (these three strains can be considered of medium CQ-resistance since the corresponding IC_{50} values for CQDP are below 100 nM). Cultures were grown in complete medium consisting of RPMI 1640 (Life Technologies, Inc.) supplemented with 11 mM glucose, 27.5 mM $NaHCO_3$, 100 UI/mL penicillin, 100 μg/mL streptomycin, and 8% heat-inactivated human serum, following the procedure of Trager and Jensen.[15] Parasites were grown at 37 °C in human A^+ red blood cells (RBCs) at a 2% haematocrit and a 3−6% parasitemia, under a 3% CO_2, 6% O_2, and 91% N_2 atmosphere. Cell cultures were synchronized by plasmagel[16] and sorbitol[17] treatments.

Increasing concentrations of various Au(I) and Au(III) complexes containing chloroquine and other ligands were tested for their inhibitory effect toward the *P. falciparum* intraerythrocytic development. The $sPLA_2$ (100 μL/well) were distributed in a 96-well plate; then 100 μL from an asynchronous culture at a 1.5% parasitemia and a 4% haematocrit in complete medium was added per well. Parasites were allowed to grow at 37 °C for 24 h in a candle jar; then 0.5 μCi of ^{3}H-hypoxanthine was added per well. After an additional 24 h incubation period, plates were freeze−thawed and harvested on filters. Dried filters were moistened in scintillation liquid mixture (OptiScint, Hisafe) and counted in a 1450 Microbeta counter (Wallac, Perkin-Elmer).

Percentage growth inhibition was calculated from the parasite-associated radioactivity. 100% ^{3}H-hypoxanthine incorporation was determined from a control grown in the absence of Au complexes. IC_{50} values were determined according to the method reported by Desjardins et al.[18] Each concentration was estimated from independent experiments in triplicate. Control culture treated with medium containing 0.16% (v/v) DMSO in the culture medium, equivalent to the maximum level of DMSO in the culture containing the complexes, did not show any inhibition of parasite growth, indicating that the observed inhibition was solely due to the presence of the drug in the medium. No effect on RBCs was observed under these conditions.

Acknowledgment. This work was supported by FONACIT (Caracas) Grant S1-98000945. The John Simon Guggenheim Foundation (New York) and the Embassy of France in Caracas are gratefully acknowledged for financial support for an exchange visit of R.A.S.-D. to MNHN Paris. Ms. Ibis Colmenares is thanked for technical assistance.

Supporting Information Available: Table S1 presenting selected ^{1}H NMR Data for Au−CQ complexes and Table 4 presenting microanalytical results. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Breman, J. G. The ears of the hippopotamus: Manifestations, determinants, and the estimates of the malaria burden. *Am. J. Trop. Med. Hyg.* **2001**, *64*, S1−S11.

(2) (a) Sánchez-Delgado, R. A.; Lazardi, K.; Rincón, L.; Urbina, J.; Hubert, A. J.; Noels, A. N. Toward a Novel Metal-Based Chemotherapy Against Tropical Diseases. 1. Enhancement of the Efficacy of Clotrimazole against *Trypanosoma cruzi* by Complexation to Ruthenium in $RuCl_2$(clotrimazole)$_2$. *J. Med. Chem.* **1993**, *36*, 2041−2043. (b) Sánchez-Delgado, R. A.; Navarro, M.; Pérez, H.; Urbina, J. Toward a Novel Metal-Based Chemotherapy Against Tropical Diseases. 2. Synthesis and Antimalarial Activity *in Vitro* and *in Vivo* of New Ruthenium− and Rhodium−Chloroquine Complexes. *J. Med. Chem.* **1996**, *39*, 1095−1099. (c) Navarro, M.; Sánchez-Delgado, R. A.; Pérez, H. 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 $[Au(PPh_3)(CQ)]PF_6$. *J. Med. Chem.* **1997**, *40*, 1937−1939. (d) Sánchez-Delgado, R. A.; Navarro, M.; Lazardi, K.; Atencio, R.; Capparelli, M.; Vargas, F.; Urbina, J. A.; Bouillez, A.; Hubert, A. J.; Noels, A. F. Toward a Novel Metal-Based Chemotherapy Against Tropical Diseases. 4. Synthesis and Characterization of new metal-clotrimazole complexes and evaluation of their activity against *Trypanosoma cruzi*. *Inorg. Chim. Acta*. **1998**, *275−276*, 528−540. (e) Navarro, M.; Lehmann, T.; Cisneros-Fajardo, E. J.; Fuentes, A.; Sanchez-Delgado, R. A.; Silva, P.; Urbina, J. A. Toward a Novel Metal-Based Chemotherapy Against Tropical Diseases. 5. Synthesis and Characterization of new Ru(II) and Ru(III) clotrimazole and ketoconazole complexes and evaluation of their activity against *Trypanosoma cruzi*. *Polyhedron* **2000**, *19*, 2319−2325. (f) Navarro, M.; Cisneros-Fajardo. E.; Lehmann, T.; Sánchez-Delgado, R. A.; Atencio, R.; Silva, P.; Lira, R.; Urbina, J. A. Toward a Novel Metal-Based Chemotherapy Against Tropical Diseases. 6. Synthesis and Characterization of new Cu(II) and Au(I) clotrimazole and ketoconazole complexes and evaluation of their activity against *Trypanosoma cruzi*. *Inorg. Chem.* **2001**, *40*, 6879−6884. (g) Navarro, M.; Vásquez, F.; Velásquez, M.; Medina, R.; Reggio, R.; Fraile, G. Effects of Gold Chloroquine Complexes on Respiratory Burst of Polymorphonuclear Leukocytes. *Arzneim.-Forsch./Drug Res.* **2002**, *52*, 468−474.

(3) Rosenberg, B. In *Nucleic Acid-Metal Ion Interactions*; Spiro, T. G., Ed.; Wiley: New York, 1980; pp 1−29. (b) Farrell, N. *Transition Metal Complexes as Drugs and Chemotherapeutic Agents, in Catalysis by Metal Complexes*; James, B. R., Ugo, R., Eds.; Kluwer: Dordrecht, The Netherlands, 1989.
(4) Sun, M. Firms Battle Over Anticancer Drug. *Science* **1983**, *222*, 145.
(5) Shaw, C. F., III. Gold- Based Therapeutic Agents. *Chem. Rev.* **1999**, *99*, 2589−2600.
(6) (a) Rhodes, M. D.; Sandler, P. J.; Scawen, M. D.; Silver, S. Effects of gold (I) Antiarthritic Drugs and Related Compounds on *Pseudomonas putida*. *J. Inorg. Biochem.* **1992**, *46*, 129−142. (b) Brown, D. H.; Smith, W. E. The Chemistry of the Gold Drugs Used in the Treatment of Rheumatoid Arthritis. *Chem. Soc. Rev.* **1980**, *9*, 217−240. (c) Coffer, M. T.; Shaw, C. F., III; Eidsness, M. K.; Watkins, J. W.; Elder, R. C. Reactions of Auranofin and (Et_3P)AuCl with Bovine Serum Albumin, *Inorg. Chem.* **1986**, *25*, 333−339. (d) Ecker, D. J.; Hempel, J. C.; Sutton, B. M.; Kirsch, R.; Crooke, S. T. Reaction of the metallodrug Auranofin [(1-Thio-β-D-glucopyranose-2,3,4,6-tetraacetato-S)-triethylphosphine gold)] with Biological Ligands. Studies by Radioisotope Methodology, *Inorg. Chem.* **1986**, *25*, 3139−3143.
(7) (a) Mirabelli, C. K.; Johnson, R. K.; Song, C. M.; Fancette, L.; Muirhead, K.; Crooke, S. T. Evaluation of the *In Vivo* Antitumor Activity and *In Vitro* Cytotoxic Properties of Auranofin, a Coordinated Gold Compound, in Murine Tumor Models. *Cancer Res.* **1985**, *45*, 32−39. (b) Köpf-Maier, P. Complexes of metals other than platinum as antitumor agents. *Eur. J. Clin. Pharmacol.* **1994**, *47*, 1−16.
(8) (a) Ferrante, A.; Kelly, B.; Scow, W.; Thong, Y. Depression of human polymorphonuclear leucocyte funtion by anti-malarial drugs. *Inmunology* **1986**, *58*, 125−130. (b) Mahoney, Ch.; Henhsey, C.; Azzi, A. Auranofin, gold thioglucose inhibit protein kinase C. *Biochem. Pharmacol.* **1989**, *38*, 3383−3386.
(9) Sundquist, W. I.; Bancroft, D. P.; Lippard, S. J. Synthesis, characterization, and biological activity of cis-diammineplatinum (II) complexes of the DNA intercators 9-aminoacridine and chloroquine. *J. Am. Chem. Soc.* **1990**, *112*, 1590−1596.
(10) Puddephatt, R. J. In *Comprehensive Coordination Chemistry*; Wilkinson, G., Gillard, R. D., McCleverty, J. A., Eds.; Pergamon: Oxford, U.K., 1987; Vol. 5, Chapter 55.
(11) Moustatih, A.; Garnier-Suillerot, A. Bifuntional Antitumor Compound: Synthesis and characterization of a Au(III)-Streptonigrin complex with thiol-modulating properties. *J. Med. Chem.* **1989**, *32*, 1426−1431.
(12) Parish, R. V.; Howe, B. P.; Wright, J. P.; Marc, J.; Pritchard, R. G.; Buckley, R. G.; Elsome, A. M.; Fricker, S. P. Chemical and biological studies of dichloro(2-((dimethylamino)methyl)phenyl)-gold (III). *Inorg. Chem.* **1996**, *35*, 1659−1666.
(13) Blackie, M. A. L.; Beagley, P.; Chibale, K.; Clarkson, C.; Moss, J. R.; Smith, P. J. Synthesis and antimalarial activity in vitro of new heterobimetallic complexes: Rh and Au derivatives of chloroquine and a series of ferrocenyl-4-amino-7-chloroquinolines. *J. Organomet. Chem.* **2003**, *688*, 144−152.
(14) Shriver, D. F.; Drezdon, M. A. *The Manipulation of Air-sensitive Compounds*, 2nd ed.; Wiley: New York, 1986.
(15) Trager, W.; Jensen, J. B. Human malaria parasites in continuous culture. *Science* **1976**, *193*, 673−675.
(16) Lambros, C.; Vanderberg, J. P. Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. *J. Parasitol.* **1979**, *65*, 418−420.
(17) Pasvol, G.; Wilson, R. J.; Smalley, M. E.; Brown, J. Separation of viable schizont-infected red cells of *Plasmodium falciparum* from human blood. *Ann. Trop. Med. Parasitol.* **1978**, *72* , 87−88.
(18) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. *Antimicrob. Agents Chemother.* **1979**, *16*, 710−718.

JM049792O