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

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Chloroquine free base (CQ) reacts with $[Rh(COD)Cl]_2$ (COD = 1,5-cyclooctadiene) and $RuCl_3 \cdot 3H_2O$/Zn to yield Rh(COD)(CQ)Cl (**1**) and $[RuCl_2(CQ)]_2$ (**2**), respectively. The two novel metal−CQ complexes, which were characterized mainly by 1D and 2D NMR spectroscopy, were tested against *Plasmodium berghei*. The *in vitro* activity of **1** was comparable to that of chloroquine diphosphate (CQDP), whereas **2** was about 5 times more active. In *in vivo* tests at equivalent concentrations of free CQ, CQDP reduced the parasitemia by 55%, while for complexes **1** and **2** the reduction reached 73% and 94%, respectively, without any sign of acute toxicity being observed up to 30 days after treatment. The Ru derivative **2** was further evaluated against two chloroquine-resistant strains of *Plasmodium falciparum*, and it was found to be 2−5 times more active than CQDP.

Malaria is probably the most important tropical disease; at present an estimated 270 million people get infected by this parasite every year, up to 2 million people die, and at least 100 million cases of acute illness are observed.[1]

Various drugs have been used for the treatment of this disease, chloroquine and sulfadoxine pyrimethamine being among the most effective. However, malaria parasites resistant to these drugs are now widespread in South America, Asia, and Africa.[2] Particulary, chloroquine-resistant strains of *Plasmodium falciparum* have become a major public health problem in all tropical areas around the world.[1,2] Hence, the search for new antimalarial therapies is a high-priority task for the control of the disease.

In our laboratories we have conceived a new strategy for the development of alternative therapies against tropical diseases, based on the modification of compounds with known or potential activity through the incorporation of a transition metal into the molecular structure. Metal complexes have been used as drugs in a variety of diseases,[3] most remarkably as antitumor agents;[3,4] however, their potential as antiparasitic agents has so far been very little explored. Some antitumor and related metal complexes have proved to be active against Trypanosomatidae protozoa,[5] and we have recently demonstrated a good activity of some metal complexes against *Trypanosoma cruzi*, the parasite responsible for Chagas disease.[6]

As part of this search for novel drugs against malaria, in this paper we report very encouraging results for two new compounds resulting from the modification of chloroquine (CQ) by coordination to metal centers (Rh, Ru); the new complexes are highly active against a chloroquine-resistant strain of *P. falciparum in vitro*, as well as against *Plasmodium berghei in vitro* and *in vivo*, in which case they appear to be nontoxic. Only a few other metal complexes have been previously tested against malaria parasites, but in contrast to our results, rather low activities or high toxicities have been found with such agents.[7]

Table 1. Selected ^{1}H NMR Data for **1** and **2**

$\Delta\delta^a$	**1**[b]	**2**[c]
H(2)	0.07	0.13
H(3)	0.04	0.17
H(5)	0.11	0.06
H(6)	0.05	0.08
H(8)	1.84	0.33
NH	0.63	0.39
H(1′)	0.05	0.15
H(2′)H(3′)	0.09	0.17
H(4′)H(5′)	0.08	0.60
H(6′)	0.05	0.16
H(1″)	0.07	0.04

[a] $\Delta\delta$ represents the displacement of the signal with respect to the corresponding one in free CQ. [b] CD_2Cl_2. [c] DMSO-d_6. Complete NMR data in Experimental Section.

Chemistry

Synthesis of RhCl(COD)(CQ) (1). Reaction of $[RhCl(COD)]_2$[8] (COD = 1,5-cyclooctadiene) with 3 equiv of CQ under mild conditions leads to the new complex RhCl(COD)(CQ) (**1**), which was isolated in good yields as air stable yellow microcrystals. Besides elemental analysis and IR spectroscopy, the new complex has been well characterized by NMR spectroscopy; all resonances could be unequivocally assigned on the basis of 1D and 2D correlated COSY and HETCOR experiments. Selected data contained in Table 1 indicate that CQ binds to the metal in **1** through the unsubstituted N(1) atom, which is a good donor site of this molecule. Correspondingly, the largest shift with respect to the free ligand is observed for H(8) ($\Delta\delta$ 1.84), located in the vicinity of N(1). All other chloroquine protons shift by <0.17 ppm except NH which moves 0.63 ppm downfield. A similar assignment of NMR data has been proposed by Lippard for the complex *cis*-$[Pt(NH_3)_2(N1\text{-}HCQ)Cl](NO_3)_2$.[9] Further support for our structural assignment came from the analysis of ^{1}H longitudinal relaxation times and

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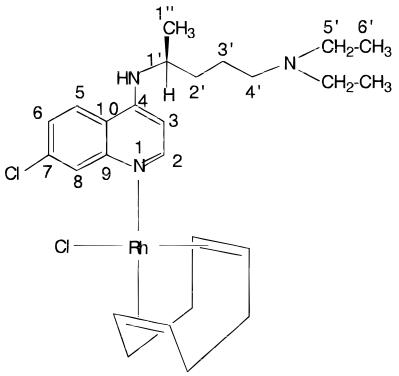
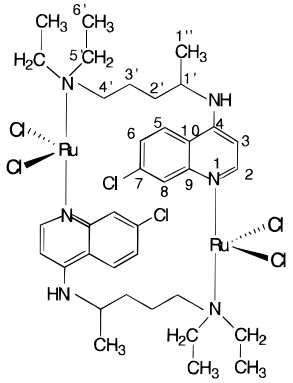
NOESY spectra which showed that the overall motion and internal flexibility of the CQ ligand in **1** were not significantly different from that of free CQ. The formulation for **1** thus corresponds to the usual 16-electron square-planar configuration for Rh(I).



(1)

Synthesis of $[RuCl_2(CQ)]_2$ (2). Interaction of $RuCl_3 \cdot 3H_2O$ with 5 equiv of CQ in the presence of Zn powder as reducing agent under mild conditions leads to the formation of a complex formulated as $[RuCl_2(CQ)]_2$ (**2**). As in the case of complex **1**, characterization has been based on microanalysis, IR, and mainly 1D and 2D NMR spectroscopy which allows assignment of all the resonances. On the basis of the selected data contained in Table 1, we believe that CQ binds to the metal in **2** through both the N(1) and N(4′) atoms, since large shifts with respect to free CQ were observed for H(8) ($\Delta\delta$ 0.33), NH ($\Delta\delta$ 0.39), and H(4′) and H(5′) ($\Delta\delta$ 0.60), while all other proton signals suffer a smaller displacement with respect to the free ligand. The proposal of a dimeric structure for complex **2** is based on the following additional detailed ^{1}H NMR studies carried out: (a) ^{1}H T_1 values for most protons of free CQ are considerably larger than the corresponding ones for complex **2**, which suggests a molecular volume of the complex of at least double that of CQ. (b) A comparison of COSY and NOESY spectra for CQ and **2** indicates that the free ligand adopts a conformation in solution in which the aliphatic protons are close in space to the all the aromatic protons, whereas in complex **2** H(2) and H(3) point away from the aliphatic chains; this can only be achieved by the interlocking of two mononuclear $[RuCl_2(CQ)]$ fragments in a single molecule through a bridging bidentate coordination of CQ, as indicated.



(2)

The proposed bonding mode of CQ in $[RuCl_2(CQ)]_2$ leads to a four-coordinated 14-electron configuration for ruthenium, which is unusual. However, in previous studies we have obtained related mononuclear Ru complexes containing N-donor ligands with similar coordination arrangements, *viz.*, $RuCl_2(CTZ)_2$[6] (CTZ = clotrimazole, 1-[(2-chlorophenyl)diphenylmethyl]-1*H*-imidazole) and $RuCl_2(BTZ)_2$[10] (BTZ = 1-[(2-bromophenyl)diphenylmethyl]-1*H*-imidazole) which has been unambiguously characterized by an X-ray diffraction study. In these cases an approximately C_{2v} arrangement of the $RuCl_2N_2$ core was found, and we believe **2** to have the same type of structure. Theoretical arguments predict such a geometry to be the most stable situation for d^6 ML_4 fragments and account for the diamagnetism observed for all three complexes.[11]

Table 2. Effect of CQDP and Complexes **1** and **2** on the *in Vitro* Growth[a] of *P. berghei*

compound	IC_{50} (nM)	IC_{50} ratio[b]
CQDP	72 ± 9	
complex **1**	73 ± 33	0.96
complex **2**	18 ± 7	4.0

[a] Growth of parasites in culture in the presence of various doses of either CQDP or compounds **1** or **2** was assayed by [^{3}H]hypoxanthine incorporation, and IC_{50} values were determined as described in the text. [b] IC_{50} ratio estimated from IC_{50}(CQDP)/IC_{50}(metal complex).

Results and Discussion

Preliminary experiments carried out to determine the *in vitro* antimalarial activities of complexes **1** and **2** were set up using *in vitro* cultures of *P. berghei*. Inhibition of the uptake of [^{3}H]hypoxanthine served as a measure of parasite viability.[12] The biological tests were carried out using DMSO as the solvent in which the complexes are stable; the NMR spectra of **1** and **2** in DMSO-d_6 remain unchanged for several days at 30 °C, showing no evidence of displacement of the CQ ligand by the solvent. Final parasitemia and hematocrit were 2% and 1%, respectively. Parasite cultures were exposed to increasing concentrations of either the parental compound or the corresponding complexes **1** and **2** for 24 h. Cells were harvested, their viability was assessed, and the concentration of drug for 50% inhibition (IC_{50}) was determined for each compound.[12] The effects of the assayed compounds on the development of *P. berghei* are summarized in Table 2. In three separate experiments the IC_{50} was estimated to be 61−81 nM for the parental compound chloroquine diphosphate (CQDP), whereas that of complex **2** was in the range of 12−26 nM. The ratio of IC_{50}(parental compound)/IC_{50}(metal complex) indicated that complex **2** was 3−5-fold more toxic to the parasite than CQDP. In contrast the same ratio for complex **1** was ≤1. Control cultures treated with medium containing 0.16% (v/v) DMSO in the culture medium, equivalent to the 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. Furthermore, neither $[Rh(COD)Cl]_2$ nor $RuCl_2(DMSO)_4$, which could conceivably be formed by loss of CQ from **1** and **2** in DMSO solution, respectively, displayed any activity against *P. berghei* in separate experiments (<0.5% inhibition).

In a further series of experiments, the *in vivo* antimalarial activity of complexes **1** and **2** was assayed in BALB/c mice infected intravenously with 10^7 erythrocytes parasitized with *P. berghei*. Test mice were

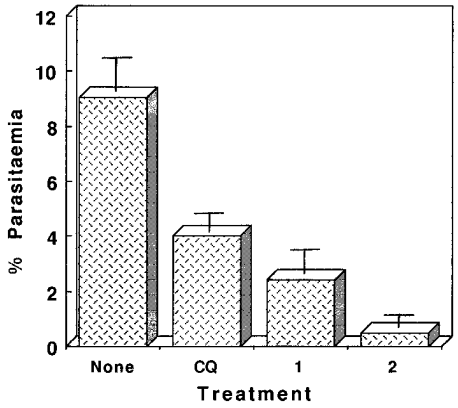


Figure 1. Effect of CQDP and complexes **1** and **2** on *P. berghei* parasitemia. The average parasitemias of nontreated control and experimental mice treated with CQDP, Rh−CQ complex **1**, or Ru−CQ complex **2** at 1 mg of CQ/kg of body weight are shown. Levels of parasitemia were determined on day 4 of infection. Vertical bars indicate one standard deviation from the mean; $n = 7$ mice/group.

infected at random before being divided into groups of seven. All compounds were administered by the intraperitoneal route; corresponding placebo was administered to control mice. Drug-treated mice received a standard[13] 4 day treatment given daily from day 0 to day 3 of innoculation. Parasitemias were counted in Giemsa-stained thin films made from tail blood on day 4. A suppressive treatment with 2.8 mg/kg CQ contained in either the parental compound or the complexes completely prevented the proliferation of parasites. Using the standard 4-day test,[13] the ED_{50} value of CQ determined for the parental compound was 1 mg/kg. Parasitemia levels were then estimated in mice receiving treatment with 1 mg/kg of CQDP or the equivalent concentration of the base in complexes **1** and **2**; these results are presented in Figure 1. Parasitaemias of mice treated with complexes **1** and **2** were significantly lower ($P < 0.01$ for **1** and $P < 0.0001$ for **2**) than that in mice treated with the parental compound as determined by the student's *t*-test; at concentrations equivalent to the ED_{50} for the parental compound, complex **1** caused a reduction of the parasitemia of 73%, while for complex **2** the reduction of parasitemia reached 94% when compared with untreated controls. Although extensive toxicity studies have not yet been carried out, it is important to point out that no lethal effect was observed in uninfected mice receiving a similar 4 day treatment (2.8 mg of CQ/kg/day) with complexes **1** or **2** up to 30 days after administration of the drugs.

These results clearly demonstrate that the metal−CQ complexes **1** and **2** are potent inhibitors of the development of the erythrocytic stages of *P. berghei* both *in vivo* and *in vitro* and *they are considerably more active than the standard parental compound and apparently nontoxic*. The increased antimalarial activity of the rhodium complex observed *in vivo* did not hold *in vitro*, the reason for this discrepancy being at present unknown. A limited water solubility in the culture medium of complex **1** probably restricted its penetration into the malaria-infected erythrocyte.

The results obtained for *P. berghei* encouraged further evaluation of the antimalarial activity of metal−CQ complexes. The more soluble Ru−CQ derivative **2**, which turned out to be the most active in the case of *P. berghei* was selected for testing[12b] using *in vitro* cultures of the FCB1 and FCB2 strains of *P. falciparum* (Colombian origin). Final parasitemia and hematocrit were 0.25−0.50% and 1.5%, respectively. The effect of complex **2** on the development of *P. falciparum* in comparison with CQDP is shown in Table 3; the IC_{50}'s reported are the average value of three experiments for FCB1 and two experiments for FCB2. In the case of FCB1, the metal complex was *about 5 times more active* than CQDP, while for FCB2, which displays a higher degree of resistance to chloroquine, *the activity was increased by a factor >2*.

Table 3. Effect of CQDP and Complex **2** on the *in Vitro* Growth of *P. falciparum*

parasite strain	IC_{50} (nM) CQDP	IC_{50} (nM) complex **2** [IC_{50} ratio][a]
FCB1	47 ± 2.3	10.5 ± 6.5 [4.48]
FCB2	104.5 ± 9.2	46.5 ± 9.0 [2.25]

[a] IC_{50} ratio estimated from IC_{50}(CQDP)/IC_{50}(metal complex).

Conclusions

Two novel chloroquine complexes of transition metals (Rh, Ru) have been synthesized by direct reactions of the free base with appropriate metal precursors. *In vitro* and *in vivo* tests against *P. berghei* showed that the incorporation of the metal fragments generally produced an enhancement of the efficacy of chloroquine; no sign of acute toxicity was observed in experimental animals after a prolonged period of time. Perhaps more importantly, the complex $[RuCl_2(CQ)]_2$ was also found to be considerably more active than chloroquine diphosphate in *in vitro* tests against chloroquine-resistant strains of *P. falciparum*. These results illustrate well the potential of the novel metal-based approach we are advancing for the development of chemotherapies against malaria and other tropical diseases.

Experimental Section

Chemistry. All manipulations were routinely carried out under N_2 using common Schlenck techniques. Solvents were purified by standard procedures immediately prior to use. Chloroquine was obtained by adding strong ammonia solution (20 mL) to chloroquine diphosphate (Sigma) (20 g, 38.9 mmol) followed by extraction with diethyl ether (200 mL). Removal of the solvent yielded an oil to which acetonitrile (30 mL) was added. On cooling to −5 °C overnight, the product precipitated as a white powder which was filtered off, washed with acetonitrile, and dried under vacuum (yield 90%). [RhCl(COD)]$_2$ was prepared according to the literature.[8] 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 carried out by Analytische Laboratorien Prof. Dr. Malissa und G. Reuter GmbH, Gummersbach, Germany.

RhCl(COD)(CQ) (1). To a solution of [RhCl(COD)]$_2$ (0.36 g, 0.73 mmol) in 2-methoxyethanol (50 mL) was added chloroquine (0.7 g, 2.2 mmol), and the mixture was refluxed under nitrogen for 4 h. The volume of the solvent was reduced to ca. 50% under a nitrogen stream, and diethyl ether was added until the solution became turbid; on cooling to −5 °C overnight, the yellow product precipitated, and it was filtered off and washed with diethyl ether and dried under vacuum: yield 65%; IR ν(N−H) 3318 cm^{-1}, ν(C=N) 1589 cm^{-1}; ν(C=C) 1611 cm^{-1}; ^{1}H NMR 6.20 (br, NH), 8.51 (d, $J = 5.93$ Hz, H2), 6.39 (d, $J = 6.32$ Hz, H3), 7.76 (d, $J = 8.64$ Hz, H5), 7.31 (d, $J = 8.44$ Hz, H6), 9.54 (s, H8), 3.68 (br, H1′), 1.31 (d, $J = 6.33$ Hz, H1″), 1.81 (m, H2′, H3′), 2.53 (m, H4′, H5′), 0.99 (t, $J = 7.14$ Hz, H6′), COD 4.61 (br, AA′), 3.55 (br, BB′), 2.53 (br, 4H), 1.59

(br, 4H); $^{13}C[^1H]$ NMR C2 152.7, C3 100.2, C4 148.0, C5 123.2, C6 125.6, C7 135.4, C8 128.7, C9 150.3, C10 118.5, C1′ 49.2, C1″ 19.9, C2′ 34.4, C3′ 24.1, C4′ 47.2, C5′ 52.9, C6′ 11.7, CH (AA′, BB′), 84.30 (d, $J_{C\text{-}Rh} = 9.43$ Hz), CH_2 31.76 (s), 30.91 (s). Anal. ($RhC_{26}H_{38}N_3Cl_2$) C, H, N; Cl: calcd, 12.50; found, 11.80.

$[RuCl_2(CQ)]_2$ (2). To a solution of $RuCl_3 \cdot 3H_20$ (0.41 g, 1.57 mmol) in MeOH (30 mL) under nitrogen were added chloroquine (2.5 g 7.8 mmol) in MeOH (20 mL) and zinc powder (0.48 g, 7.3 mmol); the mixture was refluxed under nitrogen for 6 h. The solution was filtered, and the volume of the solvent was reduced to 50% under a nitrogen stream. Diethyl ether was added until the solution became turbid; a cream-colored solid (**2**) deposited, which was filtered off, washed with methanol and diethyl ether, and dried under vacuum: yield 86%. IR ν(N−H) 3400 cm-^{1}, ν(C═C) 1600 cm^{-1}; ^{1}H NMR 7.40 (d, $J = 7.16$ Hz, NH), 8.57 (d, $J = 5.72$ Hz, H2), 6.67 (d, $J = 6.08$ Hz, H3), 8.43 (d, $J = 9.02$ Hz, H5), 7.52 (dd, $^3J = 9.04$ Hz, $^4J = 2.06$ Hz, H6), 8.23 (s, H8), 3.85 (br, H1′), 1.27 (d, $J = 6.27$ Hz, H1″), 1.62 (m, H2′), 1.57 (m, H3′), 2.95 (m, H4′), 3.00 (m, H5′), 1.12 (t, $J = 7.15$ Hz, H6′); $^{13}C[^1H]$ NMR, C2 149.8, C3 98.8, C4 150.4, C5 124.1, C6 124.5, C7 133.9, C8 126.7, C9 151.8, C1′ 47.8, C1″ 19.8, C2′ 33.3, C3′ 23.3, C4′ 46.2, C5′ 52.1, C6′ 11.5, C10 117.4. Anal. ($Ru_2C_{36}H_{52}N_6Cl_6$) C, H, N.

Biology. *In Vivo* Test for Antimalarial Activity: Animals, Parasite, and Infection. Female BALB/c mice 2−3 months old, were obtained from the breeding unit of the Instituto Venezolano de Investigaciones Cientificas. Mice were kept in plastic cages and received standard food and water *ad libitum*. The strain of *P. berghei* was obtained from Prof. Felix Pifano, Venezuelan Institute of Tropical Medicine, in 1988. In our laboratory parasites are kept frozen in liquid nitrogen as 1:2 dilution of infected blood (10−15% parasitemia) in 28% glycerol−3% sorbitol in 0.65% NaCl. To initiate infections, the 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. Chloroquine diphosphate was dissolved in 0.9% (w/v) NaCl to give the dose required in 0.1 mL for 10 g of mouse and injected intraperitoneally (ip) into mice daily for 3 consecutive days from day 0 of infection.[13] Complexes **1** and **2** were dissolved in DMSO, diluted 10-fold with intralipid (200 g of fractionated soybean, 12 g of fractionated phospholipids, 22 g of glycerol USP, 1 L of water qsp, Kavi Vitrum, Stockolm, 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 were expressed as the percentage of infected cells or inhibition of parasitemia calculated from the following equation: percent inhibition = 100 − {[estimated number of infective parasites treated with compound/estimated number of infective parasites treated with no compound (control)] × 100}.[13b] 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 (ED_{50}) of the parental compound, the percentage of erythrocytes that contain parasites of 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.

***In Vitro* Tests.** The *in vitro* activity of complexes **1** and **2** was assessed on cultures of the asexual blood stages of *P. berghei* by the method of Kamiyama[12a] and of the chloroquine-resistant FCB1 and FCB2 strains of *P. falciparum* which were derived from the Colombian FCB strain, according to the procedure described by Desjardins.[12b] Inhibition of uptake of radiolabeled nucleic acid precursor served as indicator of antimalarial activity.[12b]

Media, Cultures, and Drug Assays: **Drugs.** Chloroquine diphosphate (Sigma) was diluted in culture medium without serum/plasma to obtain a working solution of 19.4 μM. Working solutions of complexes **1** and **2** were 17.7 and 10.8 μM, respectively in RPMI medium containing 0.16% (v/v) DMSO.

***P. berghei*.** To culture parasites infected blood was obtained from BALB/c mice infected with *P. berghei* and harboring low parasitemias (3−5%) for which predominant stages were ring and young trophozoites (one nucleous). Infected blood was washed with RPMI 1640 medium (Gibco) supplemented with HEPES (25 mM), L-glutamine (0.3 g, 1−1), sodium bicarbonate (1.5 g, 1−1), and 5% normal rat serum inactivated at 56 °C/30 min. In all experiments the same batch of rat serum collected from animals 4−6 weeks old was used. Suspensions of normal and infected erythrocytes in complete culture medium were mixed to obtain a working cell suspension containing 2×10^7 parasitized cells and 1.8×10^8 uninfected erythrocytes/mL. Uninfected erythrocytes (2×10^8/mL) served as controls. Microplates were 96-well, flat-bottomed (Linbro). Twenty-five microliters of the drug solution or medium alone (with or without 0.16% DMSO) was added to the appropriate wells followed by 100 μL of the parasitized erythrocyte suspension and 25 μL of [G-^{3}H]hypoxanthine (5.4 Ci/mmol; Amersham International). A stock solution of diluted [^{3}H]hypoxanthine in culture medium was prepared[12b] to obtain a final isotope solution consisting of 20 μCi of [G-^{3}H]-hypoxanthine/mL of 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 harvested on glass-fiber disks using a cell harvester (MASH II; Microbiological Associates, MD) and counted in a liquid scintillation counter (LKB 1217 Rackbeta). The IC_{50} values of growth inhibition were calculated from the corresponding regression line. Control counts were subtracted from experimental counts, and the SD was obtained.

***P. falciparum*.** Culture medium was RPMI 1640 (Gibco) supplemented with HEPES (25 mM), sodium bicarbonate (32 mM), and human plasma 10%, v/v (O^+, collected in acid−citrate−dextrose anticoagulant), previously inactivated at 56 °C for 30 min. Stock cultures were maintained in 5 mL of 6% erythrocyte suspension in 25 mL of tissue culture flasks (Corning) at 37 °C using the candle jar method.[12d] For each experiment samples of the stock cultures were further diluted in culture medium containing sufficient noninfected human erythrocytes to yield a final hematocrit of 1.5% and parasitemia of 0.25−0.5%. Experimental cultures of *P. falciparum* contained 25 μL of the drug solution and 200 μL of the parasitized erythrocyte (O^+) suspension of either the FCB1 or the FCB2 strain. Control cultures of parasitized and nonparasitized erythrocytes (O^+) plus medium alone were included. Additional controls were parasitized erythrocytes in culture medium containing 0.16% DMSO. Microculture plates were incubated at 37 °C using the candle jar method.[12d] 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 (candle jar method) during 18 h. Cultures were then harvested and counted as indicated above. The IC_{50} values of growth inhibition were calculated from the corresponding regression line.

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References

(1) Wernsdorfer, W. H. The Development and Spread of Drug Resistant Malaria. *Parasitol. Today* **1991**, *7*, 297−303.
(2) Shapira, A.; Beales, P. F.; Halloran, M. Malaria: Living with Drug Resistance. *Parasitol. Today* **1993,** *9*, 168−174.
(3) (a) Farrell, N. Transition Metal Complexes as Drugs and Chemotherapeutic Agents. In *Catalysis by Metal Complexes*; James, B. R., Ugo, R., Eds.; Kluwer: Dordrecht, 1989. (b) Lippard, S. J., Ed. Platinum, Gold and Other Metal Chemotherapeutic Agents. *ACS Symp. Ser.* **1983**, *209*.
(4) (a) Köpf-Maier, P.; Köpf, P. Non-Platinum-Group Metal Antitumor Agents: History, Current Status and Perpectives. *Chem. Rev.* **1987**, 87, 1137. (b) Sherman, S. E.; Lippard, S. J. Structural Aspects of Platinum Anticancer Drug Interactions with DNA.

Chem. Rev. **1987**, *59*, 181−185. (c) Haiduc, I.; Silvestru, C. Metal Compounds in Cancer Chemotherapy. *Coord. Chem. Rev.* **1990**, 99, 253−296. (d) Keppler, B. K. Metal Complexes as Anticancer Agents. *New J. Chem.* **1990**, *14*, 389−403. (e) *Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy*; Howell, S. B., Ed.; Plenum: New York, 1991. (f) Sundquist, W. I.; Lippard, S. J. The Coordination Chemistry of Platinum Anticancer Drugs and Related Compounds with DNA. *Coord. Chem. Rev.* **1990**, *100*, 293−322.

(5) (a) Farrell, N. P.; Williamson, J.; McLaren, D. J. M. Trypanocydal and Antitumor Activity of Platinum-Metal and Platinum-Metal-Drug Dual-Function Complexes. *Biochem. Pharmacol* **1984**, *33*, 961−971. (b) Farrell, N.; Hacker, M. P. Properties of a Chloroquine Adduct of Rhodium Acetate. *Inorg. Chim. Acta* **1989**, *166*, 35−37. (c) Farrell, N.; Vargas, M. D.; Mascarenhas, I. A.; Gambardella, M. T. P. Rhodium Carboxylate Complexes of Diphenyltriazane and DNA-Binding ligand. Crystal and Molecular Structure of Tetrakis(μ-acetate)bis(diphenyltriazene)dirhodium(II). *Inorg. Chem.* **1987**, *26*, 1426−1429. (d) Osuna, A.; Ruiz-Pérez, L. M.; López, M. C.; Castanys, S.; Gamarro, F.; Craciunescu, D. G.; Alonso, C. Antitrypanosomal Action of cis-Diamminedichloroplatinum(II) Analogs. *J. Parasitol.* **1987,** *73*, 272−277. (e) Mesa-Valle, C. M.; Craciunescu, D.; Parrondo-Iglesias, G.; Osuna, A. In vitro Action of Platinum(II) and Platinum(IV) Complexes on *Trypanosoma cruzi and Leishmania donovani*. *Arzneim.-Forsch./Drug Res.* **1989**, *39*, 838−842. (f) Croft, S. L.; Neal, R. A.; Craciunescu, D. G.; Certad-Fombona, G. The activity of Platinum, Iridium and Rhodium Drug Complexes Against *Leishmania donovani*. *Trop. Med. Parasitol.* **1991**, *42*, 48−51. Mesa-Valle, C. M.; Moraleda-Lindez, V. ; Craciunescu, D.; Osuna, A. *In vitro* Action of New Organometallic Compounds against Trypanosomatidae protozoa. *Arzneim.-Forsch./Drug Res.* **1993**, *43*, 1010−1013.

(6) (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.

(7) Wasi, N.; Singh, H. B.; Gajanana, A.; Raichowdhary, N. Synthesis of Metal Complexes of Antimalarial Drugs and *In Vitro* Evaluation of their Activity Against *Plasmodium falciparum*. *Inorg. Chim. Acta* **1987**, *135*, 133.

(8) Herde, J. L.; Lambert, J. C.; Senoff, C. V. Di-μ-chlorobis(η^4-1,5-cyclooctadiene)dirhodium (I). *Inorg. Synth.* **1974**, *15*, 19.

(9) Sundquist, W. I.; Bancroft, D. P.; Lippard, S. J. Synthesis, Characterization and Biological Activity of cis-Diammineplatinum (II). Complexes of the DNA Intercalators 9-Aminoacridine and Chloroquine. *J. Am. Chem. Soc.* **1990**, *112*, 1590−1596.

(10) Sánchez-Delgado, R. A.; Lazardi, K.; Noels, A. N. Unpublished results.

(11) Elian, M.; Hoffmann, R. Bonding Capabilities of Transition Metal Carbonyl Fragments. *Inorg. Chem.* **1975**, *14*, 1058−1076. Seddon, E. A.; Seddon, K. R. *The Chemistry of Ruthenium*; Elsevier: Amsterdam, 1984. Schröder, M.; Stephenson, T. A. In *Comprehensive Coordination Chemistry*; Wilkinson, G.; Gillard, R. D., McCleverty, J. A., Eds.; Pergamon: Oxford, 1987; Vol. 4, Chapter 45.

(12) (a) Kamiyama, T.; Matsubara, J. Application of a simple culture of *Plasmodium berghei* for assement of antiparasitic activity. *Int. J. Parasitol.* **1992**, *22*, 1137−1142. (b) Desjardins, R.; Canfield, C.; Haynes, D.; Chulay, J. Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. *Antimicrob. Agents Chemother.* **1979**, *16*, 710−718. (c) Van Vianen, P. H.; Klaiman, D. L.; Mins, B. *Plasmodium berghei*: The antimalarial action of artemisin and sodium artelinate *in vivo* and *in vitro*, studied by flow cytometry. *Exp. Parasitol.* **1990**, *70*, 115−123. (d) Trager, W.; Jensen, J. B. Human malaria parasite in continuous culture. *Science* **1976**, *193*, 673−675.

(13) (a) Peters, W. The chemoterapy of rodent malaria, XXII. The value of drug-resistant strains of *Plasmodium berghei* screening for blood schizontocidal activity. *Ann. Trop. Med. Parasitol.* **1975**, *69*, 155−171. (b) Fujioka, H.; Yukihiro, N.; Furukawa, H.; Kumada, N. *In vitro* and *in vivo* activities of atalaphillinine and related acridone alkaloids against rodent malaria. *Antimicrob. Agents Chemother.* **1989**, *33*, 6−9. (c) Pérez, H.; De la Rosa, M.; Apitz, R. In vivo activity of ajoene against rodent malaria. *Antimicrob. Agents Chemother.* **1994**, *38*, 337−339.

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