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Synthesis, Characterization, and in vitro Antimalarial and Antitumor Activity of New Ruthenium(II) Complexes of Chloroquine

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The new Ru^{II} chloroquine complexes [Ru(η^6-arene)(CQ)Cl_2] (CQ = chloroquine; arene = *p*-cymene **1**, benzene **2**), [Ru(η^6-*p*-cymene)(CQ)$(H_2O)_2$][BF_4]$_2$ (**3**), [Ru(η^6-*p*-cymene)(CQ)(en)][PF_6]$_2$ (en = ethylenediamine) (**4**), and [Ru(η^6-*p*-cymene)(η^6-CQDP)][BF_4]$_2$ (**5**, CQDP = chloroquine diphosphate) have been synthesized and characterized by use of a combination of NMR and FTIR spectroscopy with DFT calculations. Each complex is formed as a single coordination isomer: In **1**−**4**, chloroquine binds to ruthenium in the η^1-N mode through the quinoline nitrogen atom, whereas in **5** an unprecedented η^6 bonding through the carbocyclic ring is observed. **1**, **2**, **3**, and **5** are active against CQ-resistant (Dd2, K1, and W2) and CQ-sensitive (FcB1, PFB, F32, and 3D7) malaria parasites (*Plasmodium falciparum*); importantly, the potency of these complexes against resistant parasites is consistently higher than that of the standard drug chloroquine diphosphate. **1** and **5** also inhibit the growth of colon cancer cells, independently of the p53 status and of liposarcoma tumor cell lines with the latter showing increased sensitivity, especially to **1** (IC_{50} 8 μM); this is significant because this type of tumor does not respond to currently employed chemotherapies.

Introduction

The success of cisplatin and other platinum anticancer drugs has stimulated a renaissance of inorganic medicinal chemistry and the search for complexes of other transition metals with interesting biological properties.[1−3] Ruthenium complexes are attracting increasing attention as potential chemotherapeutic agents against a variety of diseases. The coordination chemistry of ruthenium has been extensively

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developed, particularly with ligands of biological relevance; the preferred octahedral coordination for the common +2 and +3 oxidation states in aqueous solution, together with adequate substitution rates and redox potentials for biological interactions and a demonstrated low toxicity make Ru a particularly attractive choice for the development of new metallopharmaceuticals.[1−4] Most of the effort has been directed toward anticancer agents, with notable examples reaching phase I clinical trials, such as the complex [imH] *trans*-[$RuCl_4$(Im)(dmso)] (NAMI, Im = imidazole), which is active against metastases[5] and [IndH] *trans*-[$RuCl_4(Ind)_2$] (KP1019, Ind = indazole), which has a high activity against colon cancer.[6] Ru^{II} complexes containing the arene/ethylenediamine ligand combination and related derivatives[7] as well as Ru^{II}−arene−PTA complexes (PTA = 1,3,5-triaza-7-phosphaadamantane)[8] also display very good antitumor activity.

An alternative approach to the discovery of new metallodrugs involves binding an organic compound of known therapeutic value to a metal-containing fragment; this results in a metal−drug synergism in which the metal acts as a carrier and stabilizer for the drug until it reaches its target, while at the same time the organic drug carries and protects the metal, preventing side reactions in its transit toward a second target of biological action.[3] Such combined effects may result in an important enhancement of the activity of the drug and the opening of new mechanisms of action, such as when the well-known breast cancer drug tamoxifen is coordinated to iron (Ferrocifen), or to a lesser degree, to ruthenium.[9] We have successfully applied this concept in our search for novel antiparasitic agents: Ru^{II} and Ru^{III} complexes of two commonly employed imidazole-based fungicides, clotrimazole (CTZ) and ketoconazole (KTZ), have proved to be highly active against *Trypanosoma cruzi*, the causative agent of Chagas disease, an essentially incurable and highly prevalent ailment in Latin America.[10] The free azole ligands or Ru^{II} complexes not containing CTZ or KTZ have only a marginal activity against this parasite; only the combination of both in a single molecule produces a high activity due to a dual target mechanism involving sterol biosynthesis inhibition at the parasite's membrane by CTZ or KTZ, together with selective Ru−DNA binding.[11] Interestingly, we showed that some of these complexes also display anticancer activity, notably $RuCl_2(KTZ)_2$, which resulted more active than cisplatin against several types of tumor, acting by a different mechanism, something of interest in relation to drug resistance.[12]

We have also followed this approach in the search for new antimalarial agents. Malaria continues to be one of the most serious health problems affecting humans in vast regions of the planet; over 300−500 million people become infected and close to one million die of malaria each year, mostly children under 5 years.[13] Although progress has been made over the last two decades toward antimalarial vaccines, it is unlikely that eradication of malaria through vaccination will become a global reality in the short term.[14] The most serious problem in the treatment of malaria is that the parasites causing the disease have developed strong resistance to the most widely used drugs, particularly chloroquine (CQ), which was the treatment of choice for several decades.[13] Therefore, new chemotherapeutic agents are urgently needed, particularly against drug-resistant strains of *Plasmodium falciparum*, the most deadly form of the parasite; metal-based drugs containing the chloroquine moiety are emerging as promising alternatives (Chart 1). In previous work we demonstrated that coordinating CQ to simple metal-containing fragments (Ru,[15,16] Rh,[15] and Au[17]) leads to enhanced activity against CQ-resistant parasites; similar Ir−CQ derivatives have also been reported to be active in vivo against rodent malaria.[18]

Of particular relevance to this work, our ruthenium complex $[RuCl_2(CQ)]_2$ (**A** in Chart 1) displayed enhanced activity in vitro against CQ-resistant FCB1 and FCB2 strains of *P. falciparum* and in vivo against *Plasmodium berghei*

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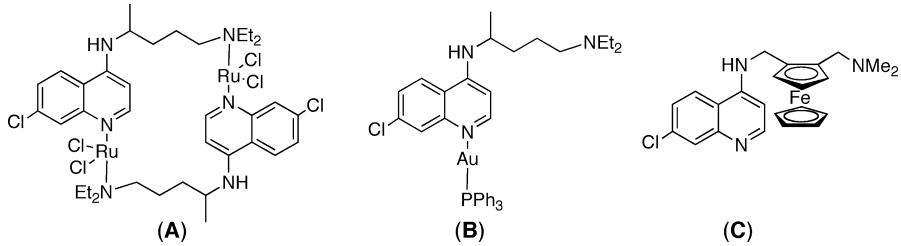
Chart 1. Examples of Metal Derivatives of Chloroquine with Antimalarial Properties



(rodent malaria);[15] the mechanism of action of this complex has been investigated in detail and the role of the metal fragment has been shown to be an alteration of the structure, the basicity, and most importantly the lipophilicity of CQ to make it less recognizable to the parasite's defense mechanism.[16] A different approach to metal-based antimalarials involves the use of chloroquine-like molecules containing an organometallic fragment covalently linked to the side chain. A notable example by Biot, Brocard, and co-workers is ferroquine, a modified CQ molecule containing a ferrocenyl unit (**C** in Chart 1).[19] This compound is highly active and specific against CQ-resistant *P. falciparum* and it is currently in clinical development; the ruthenium analog (ruthenoquine) was also synthesized and its biological activity was similar to that of the iron derivative.[19] Moss and Chibale have extended this chemistry through the synthesis of a number of CQ derivatives containing ferrocenyl or ruthenocenyl units and a variety of other substituents in the side chain of the molecular structures. These compounds are also active against CQ-sensitive and CQ-resistant *P. falciparum*, with no significant differences in efficacy between the two metals.[20]

A further point of interest is that CQ has been shown to display some anticancer activity[21] or preventive effect[22] and to produce an enhancement of the potency of other known drugs.[23] We therefore reasoned that because both Ru^{II} and CQ can be associated with anticancer effects, a combination of both in a single molecule would lead to interesting antitumor properties. The relatively simple structure of our first-generation compound $[RuCl_2(CQ)]_2$, which contains only CQ and hydrolyzable chloride ligands,[15] does not lend it amenable to much molecular manipulation to further enhance the biological activity. Thus, we adopted a new molecular design in our search for novel metal-based chemotherapeutic agents against malaria and cancer that makes use of the $Ru^{II}(\eta^6\text{-arene})(CQ)L_2$ structure, which allows for variation of the ancillary ligands and the overall charge in the complexes; $(\eta^6\text{-arene})Ru^{II}$ structures with various other ligands have proved useful in the development of effective anticancer agents by other workers, notably Sadler[7] and Dyson.[8] In this article, we report the synthesis of a series of new organo-Ru^{II}−CQ complexes and their characterization by a combination of spectroscopic and theoretical methods, together with data on their biological activity, which demonstrates that the combination of Ru^{II} and chloroquine in the same molecular structure, stabilized by arene ligands, results in enhanced activities against resistant malaria parasites and against certain types of cancer cells.

Experimental Section

All manipulations were carried out under N_2 using common Schlenk techniques. Solvents (analytical grade, Aldrich) were dried and degassed immediately prior to use by means of an Innovative Technology solvent purification unit; ruthenium trichloride hydrate (Pressure Chemicals, Inc.), chloroquine diphosphate, and other reagents (Aldrich) were used as received. $[Ru(p\text{-cymene})Cl_2]_2$,[24] $[Ru(\eta^6\text{-benzene})Cl_2]_2$,[24] and $[Ru(p\text{-cymene})Cl(en)]PF_6$[25] were prepared according to published procedures. Elemental analyses were performed by Atlantic Microlab, Norcross, Georgia. FTIR spectra were measured on a Thermoelectron NICOLET 380 FTIR spectrometer. Conductivity values were obtained using 1 mM solutions of the complexes in water or other appropriate solvents at various time intervals using an Oaklon pH/Conductivity meter. NMR spectra were obtained using an AVANCE Bruker 400 instrument; chemical shifts are relative to residual proton (or carbon) signals in the deuterated solvents. The atom numbering for chloroquine is shown below; all peaks were unambiguously assigned by use of a combination of 1D and 2D (COSY, HSQC, and HMBC) experiments:

Chloroquine Base (CQ). CQ was obtained by a modified version of a published procedure.[15] Concentrated ammonia solution (20 mL) was added to chloroquine diphosphate (CQDP) (20 g, 38.9 mmol) followed by two extractions with chloroform (200 mL). Removal of the solvent yielded an oil to which acetonitrile (30 mL) was added. The solution was evaporated to dryness to yield a

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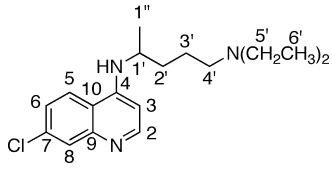

white powder, which was washed with acetonitrile (5 mL) and diethyl ether (5 mL) and dried under vacuum.

[Ru^{II}(η^6-p-cymene)Cl$_2$(CQ)] (1). [Ru(η^6-*p*-cymene)Cl$_2$]$_2$ (612 mg, 1 mmol) and chloroquine base (640 mg, 2 mmol) were stirred in acetone (30 mL) under N_2 for 4 h at room temperature. The orange suspension was evaporated to dryness to yield an orange solid, which was dissolved in water. The yellow aqueous solution was filtered through Celite and evaporated to dryness to yield a solid that was dried under vacuum. Yield = 96%. ^{1}H NMR (D_2O) δ (ppm): 8.25 (d, J = 6.73 Hz, 1H); 8.09 (d, J = 9.11 Hz, 1H); 7.75 (d, J = 2.05 Hz, 1H); 7.51 (dd, J = 2.08 Hz, J' = 9.09 Hz, 1H); 6.72 (d, J = 6.85 Hz, 1H); 5.32 (d, J = 6.21 Hz, 2H); 5.13 (d, J = 6.21 Hz, 2H); 4.00 (m, 1H); 3.12 (m, 6H); 2.62 (m, 1H); 2.03 (s, 3H); 1.76 (br, 4H); 1.33 (d, 3H); 1.18 (d, 6H); 1.16 (m, 6H). ^{13}C NMR (D_2O) δ (ppm), C(4) 153.87, C(2) 144.42, C(9) 140.22, C(7) 136.01, C(6) 126.60, C(5) 123.69, C(8) 120.59, C(10) 115.37, C(3) 98.76, C(F) 98.49, C(G) 92.74, C(E) 76.47, C(D) 75.94, C(4′) 51.45, C(1′) 49.14, C(5′) 47.48, C(2′) 32.05, C(C) 30.51, C(6′) 21.93, C(3′) 20.42, C(1″) 18.98, C(B) 17.67, C(A) 8.33. Anal. Calcd for $C_{28}H_{40}N_3Cl_3Ru\cdot H_2O$: C, 52.17; H, 6.52; N, 6.52. Found: C, 52.48; H, 6.48; N, 6.64.

[Ru^{II}(η^6-benzene)Cl$_2$(CQ)] (2). [Ru(η^6-benzene)Cl$_2$]$_2$ (250 mg, 0.5 mmol) was suspended in acetonitrile (30 mL) under N_2. The mixture was stirred at room temperature until all the solid dissolved to form a dark-brown solution. Chloroquine base (320 mg, 1.0 mmol) was then added and the resulting solution was stirred at room temperature for 20 h. A dark beige-green solid formed, which was filtered off and dried under vacuum. The product was purified by stirring the solid in 20 mL of acetone for 1 h after which it was filtered off and dried under reduced pressure. Yield: 70% ^{1}H NMR (MeOD) δ (ppm) 8.33 (d, 1H, J = 8.80 Hz); 8.26 (d, 1H; J = 6.40 Hz); 7.71 (d, 1H; J = 2.00 Hz); 7.43 (dd, 1H; J = 8.90 Hz, J' = 2.00 Hz); 6.68 (d, 1H; J = 6.40 Hz); 5.38 (s, 6H); 3.94 (m, 1H); 3.07 (m, 6H); 1.76 (m, 4H); 1.29 (d, 3H; J = 6.40 Hz); 1.18 (t, 6H; J = 7.20 Hz). ^{13}C NMR (MeOD) δ (ppm) C(4) 153.30, C(2) 146.79, C(9) 143.59, C(7) 137.36, C(6) 125.94, C(5) 124.24, C(8) 122.52, C(10) 116.55, C(3) 98.67, C(A) 77.30, C(4′) 51.61, C(1′) 48.88, C(5′) 45.88, C(2′) 32.39, C(3′) 20.81, C(1″) 18.77, C(6′) 7.82. Anal. Calcd for $C_{24}H_{32}N_3Cl_3Ru$: C, 50.58; H, 5.66; N, 7.37. Found: C, 50.51; H, 5.84; N, 7.55.

[Ru^{II}(η^6-p-cymene)(H$_2$O)$_2$(CQ)][BF$_4$]$_2$ (3). [Ru(η^6-*p*-cymene)Cl$_2$]$_2$ (400 mg, 0.653 mmol) and $AgBF_4$ (509 mg, 2.614 mmol) were stirred in acetone (40 mL) for 2 h at 55 °C under N_2. After cooling to room temperature, the solution was filtered through celite to remove AgCl. Chloroquine base (418 mg; 1.307 mmol) was added to the filtrate and the mixture was allowed to react at 55 °C for 20 h and then cooled to ambient temperature. The resulting reddish-brown-colored solution was dried under vacuum to obtain a brown solid, which was stirred in pentane (40 mL) overnight. The resulting brownish-yellow-colored solid was filtered off under nitrogen and dried under vacuum. Yield 96%. 1H NMR (MeOD) δ (ppm) 8.255 (d, 1H); 8.231 (d, 1H); 7.719 (s, 1H); 7.462 (dd, J = 9.030 Hz, J' = 7.456 Hz, 1H); 6.715 (d, J = 6.179 Hz, 1H); 5.315 (d, J = 5.258 Hz, 2H); 5.111 (d, J = 5.274 Hz, 2H); 3.958 (m, 1H); 3.101 (m, 4H); 3.065 (m, 2H); 2.68 (m, 1H); 2.092 (s, 3H); 1.74 (m, 2H); 1.71 (m, 2H); 1.3 (d, J = 6.089 Hz, 3H); 1.197 (d, 6H); 1.152 (t, 6H). ^{13} C NMR (MeOD) δ (ppm) C (4) 153.343, C (2) 146.62, C (9) 143.296, C (7) 137.448, C (6) 126.02, C (5) 123.91, C (8) 122.318, C (10) 116.42, C (3) 98.674, C (F) 96.546, C(G) 91.773, C(E) 77.191, C(D) 76.033, C (4′) 51.551, C(C) 28.156, C(B) 16.948, C(1′) 48.779, C (5′) 47.197, C (2′) 32.266, C (3′) 20.662, C (1″) 18.672, C(A) 21.322 C (6′) 7.812. Anal. Calcd for $C_{28}H_{42}N_3ClORuB_2F_8$ C, 45.08; H, 5.63; N, 5.63. Found: C, 45.09; H, 5.88; N, 5.52.

[Ru^{II}(η^6-p-cymene)(en)(CQ)][PF$_6$]$_2$ (4). Chloroquine base (67.3 mg, 0.210 mmol) and $AgPF_6$ (53.1 mg, 0.210 mmol) were added to a solution of [Ru(η^6-*p*-cymene)Cl(en)][PF_6] (100 mg, 0.210 mmol) in dry methanol (60 mL). The mixture was protected from light and left to react for 20 h under N_2 at room temperature. The final solution was filtered and methanol was removed under vacuum. The product was extracted with acetone to remove the excess of AgCl and then diethyl ether was added to promote the precipitation of a red-orange solid, which was filtered and dried under vacuum. Yield: 70%. ^{1}H NMR (MeOD) δ (ppm) 8.386 (b, 1H); 8.293 (d, 1H; J = 8.80 Hz); 7.811 (s, 1H); 7.500 (dd, J = 9.20 Hz, J' = 1.60 Hz, 1H); 6.730 (d, J = 6.02 Hz, 1H); 5.890 (b, NH_2); 5.626 (d, J = 6.42 Hz, 2H); 5.458 (d, J = 6.41 Hz, 2H); 4.010 (m, 1H); 3.174 (m, 4H); 2.881 (m, 1H; J = 7.20 Hz); 2.549 (m, 2H); 2.448 (m, 2H); 2.239 (s, 3H); 1.857 (m, 4H); 1.402 (d, 3H; J = 6.42 Hz); 1.301 (d, 6H; J = 6.82 Hz); 1.260 (t, 6H; J = 6.41 Hz). ^{13}C NMR (MeOD) δ (ppm) C(4) 153.644, C(2) 149.98, C(9) 146.898, C(7) 137.831, C(6) 126.841, C(8) 125.391, C(5) 124.959, C(10) 118.272, C(F) 105.817, C(3) 100.106, C(G) 98.554, C(D) 83.600, C(E) 81.843, C(4′) 53.066, C(1′) 49.672, C(5′) 45.949, C(en) 45.556, C(2′) 33.845, C(C) 32.009, C(A) 22.776, C(3′) 22.179, C(1″) 20.199, C(B) 18.146, C(6′) 9.276. Anal. Calcd for $C_{30}H_{48}N_5ClRuP_2F_{12}$ C, 39.80; H, 5.35; N, 7.78. Found: C, 39.60; H, 5.37; N, 7.72.

[Ru^{II}(η^6-p-cymene)(η^6-CQDP)][BF$_4$]$_2$ (5). [Ru(η^6-*p*-cymene)Cl$_2$]$_2$ (300 mg, 0.490 mmol) was dissolved in warm deionized water (40 mL, 55 °C); $AgBF_4$ (382 mg, 1.96 mmol) and chloroquine diphosphate (506 mg, 0.98mmol) were added under nitrogen. The mixture was stirred for 20 h at 55 °C and then filtered through celite to yield an orange solution. The solvent was evaporated and the final product was dried under vacuum for 20 h. Yield 78%. ^{1}H NMR (D_2O) δ (ppm) 8.183 (d, J = 7.246 Hz, 1H); 8.130 (d, J = 9.119 Hz, 1H); 7.764 (d, J = 1.942 Hz, 1H); 7.542 (dd, J = 9.125 Hz, J' = 1.978 Hz, 1H); 6.748 (d, 7.307 Hz, 1H); 5.847 (d, J = 6.3 Hz, 2H); 5.627 (d, J = 6.22 Hz, 2H); 4.033 (m, 1H); 3.065 (m, 6H); 2.781 (m, 1H); 2.137 (s, 3H); 1.717 (m, 4H); 1.288 (d, J = 6.474 Hz, 3H); 1.243 (d, J = 6.942 Hz, 6H); 1.128 (td, J = 7.152 Hz, J' = 1.912 Hz, 6H).^{13}C NMR (D_2O) δ (ppm) C(4)155.639, C(2) 142.265, C(7) 139.388, C(9) 138.36, C(6) 127.408, C(5) 124.177, C(8) 119.254, C(10) 115.436, C(F) 99.734, C(3) 98.527, C(G) 96.075, C(E) 79.034, C(D) 76.245, C(5′) 51.281, C(1′) 49.518, C(4′) 47.43, C(C) 30.725, C(A) 21.288, C(2′) 31.987, C(3′) 20.305, C(1″) 17.703, C(B) 17.703, C(6′) 8.196. Anal. Calcd for $C_{28}H_{46}N_3ClO_8P_2RuB_2F_8.2H_2O$ C, 34.96; H, 5.20; N, 4.37. Found: C, 35.04; H, 4.94; N, 4.37.

DFT Calculations. All calculations reported in this study were carried out by use of the *Gaussian 03* program package.[26] All molecular structures, frequencies, and normal-mode composition were computed using the B3LYP density functional in combination with a LANL2DZ effective core potential for ruthenium[27] and moderate 6-31G(d) basis sets for all remaining atoms. Computed frequencies of all structures are positive, indicating that the structures are at real minima of their ground-state potential energy surfaces. The relative energies of structural isomers were estimated based on full optimizations employing a gas-phase model and the

polarizable continuum model (PCM)[28] to mimic electrostatic effects of aqueous solutions.

Antimalarial Activity Measurements. The following strains of *Plasmodium falciparum* were employed in this study: FcB1 (Colombia), PFB (Brazil), F32 (Tanzania), W2 (Indonesia), Dd2 (SE. Asia), K1 (SE. Asia), and 3D7 (origin unknown). In our routine culture conditions, that is in the absence of chloroquine pressure, FcB1, PFB, 3D7, and F32 were chloroquine-sensitive (IC_{50} < 100 nM), whereas W2, Dd2, and K1 were chloroquine-resistant (IC_{50} > 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 8−10% heat-inactivated human serum, following the procedure of Trager and Jensen.[29] Parasites were grown at 37 °C in human A^+ (FcB1, PFB, F32) or O^+ (W2, Dd2, K1, 3D7) red blood cells at a 2% hematocrit and a 2−6% parasitemia, under a 3% CO_2, 6% O_2, and 91% N_2 atmosphere. W2, Dd2, K1, and 3D7 parasites were synchronized by sorbitol[30] treatment.

According to their respective solubility in H_2O, 1 mM stock solutions of the Ru complexes **1**−**3** and **5** were prepared in either H_2O or 10% DMSO (Ru-complex **3**). Further dilutions were in complete culture medium. The complexes were tested for their inhibitory effect toward the *P. falciparum* intraerythrocytic development. Increasing concentrations of the complexes and chloroquine (100 μL/well, top concentration = 50 μM) were distributed in a 96 well microplate; DMSO (0.2% vol/vol, top concentration) was distributed for control. Then, for the FcB1, PFB, and F32 strains, 100 μL from an asynchronous culture at a 1.0% parasitaemia and a 4.0% hematocrit 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, and the culture was incubated for an additional 24 h period. For the K1, Dd2, 3D7, and W2 strains, 100 μL from a culture containing >90% rings (age 0−20 h postinvasion) at a 0.5−1.0% parasitaemia and a 3.0% hematocrit in complete medium was added per well along with 1.0 μCi of ^{3}H-hypoxanthine. Parasites were grown for 42 h at 37 °C in a candle jar. 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, PerkinElmer). 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 Ru complexes. IC_{50} values were determined according to the method reported by Desjardins et al.[31]

Antitumor Activity Measurements. (i) Cell Culture. LS141 primary human cell line was derived from a patient with high-grade retroperitoneal dedifferentiated liposarcoma.[32] Cells were grown in RPMI 1640 supplemented with 15% fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin, and maintained at 37 °C in 5% CO_2. HCT-116 human colon carcinoma cell line, which is wild-type for p53 ($p53^{+/+}$) and its p53-null ($p53^{-/-}$) variant were a generous gift from Dr. B. Vogelstein (J. Hopkins University, Baltimore, MA). Cells were grown in McCoy's supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin and maintained at 37 °C in 5% CO_2.

(ii) Colorimetric Cell Proliferation Assay. The assay was performed as per the manufacturer's protocol (Dojindo Molecular Technologies, Inc., Gaithersburg, MD). Briefly, 1500 cells were plated in 100 mL volume in each well of a 96 well plate and treatments were done 24 h after plating. After incubation for 72 h with the drug, 20 μL of CCK-8 solution were added to each well and were further incubated at 37 °C for 1−4 h. Then the OD at 450 nm to determine the cell viability was measured using Spectra Max 340 PC (Molecular Devices Corp, Sunnyvale CA). IC_{50}'s were defined as concentrations of the complexes that inhibited proliferation by 50% relative to untreated controls. These experiments were carried out in at least triplicate.

Results and Discussion

Synthesis of New Complexes. The well-known dimers $[Ru(\eta^6\text{-arene})Cl_2]_2$ (arene = *p*-cymene or benzene) are convenient precursors to new Ru^{II} complexes containing CQ, according to the set of reactions represented in Scheme 1; elemental analyses, as well as ^{1}H and ^{13}C NMR data are fully consistent with the proposed formulations (Experimental Section). A bridge-splitting reaction of the *p*-cymene dimer with CQ in acetone affords the neutral dichloro species $Ru(\eta^6\text{-}p\text{-cymene})Cl_2(CQ)$ (**1**) in high yield.

Conductivity measurements indicate that **1** remains neutral in chloroform solution (nonconducting) but it rapidly exchanges one chloride ligand by a solvent molecule in polar solvents to reach conductivities of 47 $\mu S \cdot cm^{-1}$ in methanol and 100.5 $\mu S \cdot cm^{-1}$ in water; the conductivity values do not vary over a period of 24 h. In aqueous solution, the only Ru-containing species present within 1 min of dissolution is thus the monocationic derivative $[Ru(\eta^6\text{-}p\text{-cymene})Cl(H_2O)(CQ)]^+$ (**1′**) and no NMR spectral changes consistent with a recoordination of a chloride are observed upon addition of up to 2 equiv of sodium chloride. Aqueous solutions of **1′** are stable over prolonged periods of time in the air, something important in relation to possible biological applications. An analogous reaction of the benzene dimer in acetonitrile yields the corresponding product $Ru(\eta^6\text{-benzene})Cl_2(CQ)$ (**2**), which also dissociates one chloride ligand rapidly in water to form the corresponding monocationic derivative $[Ru(\eta^6\text{-benzene})Cl(H_2O)(CQ)]^+$ (**2′**) (conductivity 108 $\mu S \cdot cm^{-1}$). On the other hand, the dicationic

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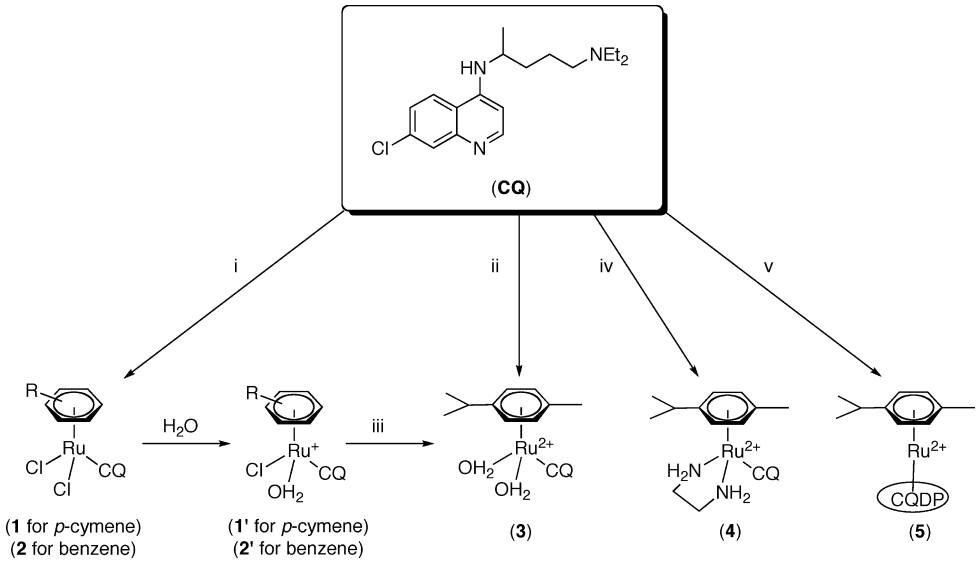
Scheme 1. Synthesis of New Ru−Chloroquine Complexes[a]



[a] (i) $[Ru(\eta^6\text{-arene})Cl_2]_2$ (arene = *p*-cymene, Me_2CO, 25 °C; benzene, acetonitrile, 25 °C); (ii) $[(Ru\ \eta^6\text{-}p\text{-cymene})Cl_2]_2/AgBF_4$ (1:4), Me_2CO, 55 °C, **3** isolated as the BF_4 salt; (iii) $AgBF_4$ (1:1), Me_2CO, 55 °C, **3** isolated as the BF_4 salt; (iv) $[Ru(\eta^6\text{-}p\text{-cymene})Cl(en)][PF_6]/AgPF_6$ (1:1), MeOH, 25 °C, **4** isolated as the PF_6 salt; (v) CQ used as the diphosphate salt CQDP, $[Ru(\eta^6\text{-}p\text{-cymene})Cl_2]_2/AgBF_4$ (1:4), H_2O, 55 °C, **5** isolated as the BF_4 salt.

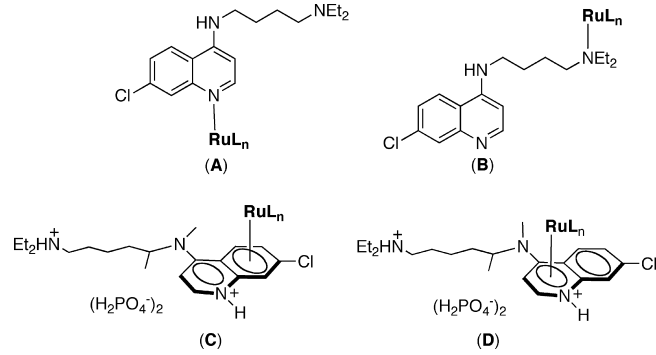
complex $[Ru(\eta^6\text{-}p\text{-cymene})(H_2O)_2(CQ)][BF_4]_2$ (**3**) is best obtained in a one-pot procedure by treatment of $[Ru(\eta^6\text{-}p\text{-cymene})Cl_2]_2$ with 4 equiv of $AgBF_4$ at 55 °C, followed by reaction with CQ at the same temperature; alternatively, it may be prepared by reaction of [**2′**]BF_4 with 1 equiv of $AgBF_4$. The Ru^{II} ethylenediamine complex $[Ru(\eta^6\text{-}p\text{-cymene})Cl(en)]^+$ displays good in vitro anticancer activity.[7] Removal of the chloride from this complex with 1 equiv $AgPF_6$ in the presence of CQ leads to the dicationic complex $[Ru(\eta^6\text{-}p\text{-cymene})(CQ)(en)]^{2+}$ (**4**). This compound is stable as a solid and in aqueous or methanol solutions. The conductivity of a 1 mM solution of **4** in water is 265 $\mu S \cdot cm^{-1}$, in agreement with the proposed dicatonic structure.

Chloroquine diphosphate (CQDP) is the standard form of the drug used in antimalarial treatment. In this salt, the two more basic nitrogen atoms at the quinoline and at the diethylamino group are protonated and therefore unavailable for coordination to the metal. Reaction of CQDP with $[(\eta^6\text{-}p\text{-cymene})Ru(solv)_3]^{2+}$, generated by removal of the chloride ligands from $[Ru(\eta^6\text{-}p\text{-cymene})Cl_2]_2$ with $AgBF_4$ in water, leads in this case to an unprecedented π-coordination of the bioactive ligand to Ru through an aromatic ring and formation of the 18 electron sandwich compound $[Ru(\eta^6\text{-}p\text{-cymene})(\eta^6\text{-CQ})][BF_4]$ (**5**).

Bonding Mode of the CQ Ligand. The biological activity of CQ is strongly dependent on structural and physicochemical features. In particular, basicity and lipophilicity are key properties for the accumulation of the drug in the acidic food vacuole of malaria parasites[13] or in acidic regions surrounding tumors.[22] The quinoline and diethylamino nitrogen atoms are the most basic sites of this molecule and therefore they are the likely sites for metal binding. Chart 2 summarizes the possible coordination modes of CQ and CQDP to $Ru(\eta^6\text{-}p\text{-cymene})$ fragments.

Examples of CQ binding to metals through the nitrogen atom of the quinoline ring (**A**)[15] or the one in side chain

Chart 2. Possible Coordination Modes of CQ and CQDP in Ru Complexes



(**B**)[18] are known. In the case of CQDP, those two nitrogen atoms are protonated and therefore coordination to Ru must involve π-bonding to either the carbocyclic (**C**) or the heterocyclic (**D**) ring of the quinoline moiety; to our knowledge, there are no previous examples of complexes containing π-bonded CQDP ligands.

In any one of the possible binding modes, coordination to the metal will have an important effect on the basicity and lipophilicity of CQ and it clearly represents a major structural modification of the organic drug. Although the mechanisms of CQ-resistance are not entirely clear, it is generally accepted that drugs with increased lipophilicity are more efficient in overcoming resistance[33] and that the mutated protein responsible for resistance is highly structure specific.[13c,16] It is therefore important to ascertain the coordination mode of CQ to Ru in each new complex to understand how such structural variations are reflected in different biological properties. Repeated attempts to obtain

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X-ray quality crystals of the new Ru−CQ derivatives have so far proved unfruitful and in fact no structure of a metal complex containing coordinated CQ has been published as yet.[34] We therefore rely on a combination of NMR experiments and DFT calculations together with FTIR data to determine the binding mode of CQ to Ru in our new complexes.

NMR Studies. We[16−18] and others[35] have argued that the largest variations in the ^{1}H and ^{13}C chemical shifts ($\Delta\delta$) of a bound ligand with respect to the values for the free form are observed for the nuclei in the close vicinity of the coordination site and that this may therefore serve as a diagnostic tool for determining binding preferences of bioactive ligands. Table S1 of the Supporting Information summarizes the most relevant $\Delta\delta$ data for **1**−**5**. We note that for this series of compounds the $\Delta\delta$ criteria generally hold for the ^{13}C spectra but some ambiguities emerge in the case of the ^{1}H data. For **1**, the largest value of ^{13}C $\Delta\delta$ is for C2, adjacent to the nitrogen atom in the heterocyclic ring, indicating coordination of CQ to Ru through the quinoline nitrogen (**A**); in agreement with this, the largest ^{1}H $\Delta\delta$ values are observed for H2 and H8. In the case of **2**, large ^{13}C $\Delta\delta$ values are observed for the aromatic carbons C(2) and C(8) as expected for structure **A** but H(4′) and H(5′), located in the vicinity of diethylamino group, experience a larger shift than the aromatic H(2) or H(8), which could suggest binding of the metal to the diethylamino group as in **B**. For dicationic **3**, the ^{13}C $\Delta\delta$ values again point to coordination at the quinoline nitrogen (**A**), whereas the ^{1}H $\Delta\delta$ data suggests binding to the side chain (**B**); similarly, ethylenediamine derivative **4** displays ^{13}C $\Delta\delta$ values indicative of coordination at the quinoline and ^{1}H $\Delta\delta$ values consistent with binding at the side chain. In the case of π-bonded **5**, both the ^{1}H and the ^{13}C $\Delta\delta$ values are consistent with binding at the carbocyclic (**C**), rather than the heterocyclic ring (**D**). The ^{13}C $\Delta\delta$ data are thus generally more coherent than the ^{1}H $\Delta\delta$ data and indicate binding at the ring nitrogen (**A**) for **1**−**4**; this is the most common isomeric form proposed for other metal complexes containing coordinated CQ.[16−18]

To develop a more reliable means of determining coordination preferences, we measured the spin−lattice relaxation times T_1 for all ^{1}H signals of the new complexes and compared them with those of free CQ. T_1 relaxation measures the rate at which the spin system comes into thermal equilibrium with the other degrees of freedom and it involves interactions of the nucleus being observed with its surroundings;[36] therefore, T_1 magnitudes are very sensitive to electronic and structural perturbations, such as the one caused by coordination of an organometallic fragment to the molecule under study. A particularly marked variation in the T_1 values with respect to the free ligand (ΔT_1) is thus expected for protons located in the vicinity of the Ru−N bond. The data in Table 1 show that the ΔT_1 values for **1**−**4** are indeed much larger for the aromatic protons, particularly for H2 and H8, than for the aliphatic protons of the side chain, consistently indicating that CQ binds to Ru through the quinoline nitrogen in **1**−**4**, in agreement with the trend of ^{13}C $\Delta\delta$ values and with most previous assignments of CQ coordination to metals;[16−18] it is reasonable to expect that all members of this series of related complexes display the same bonding preference of CQ.

Table 1. ΔT_1 (s) for Ru−CQ Complexes[a]

proton	1	2	3	4	5
H2	**1.59**	**0.48**	**1.35**	[b]	**0.49**
H3	0.67	0.01	0.14	0.22	0.00
H5	0.35	0.38	0.88	1.04	**0.40**
H6	1.32	0.11	0.72	0.87	**0.38**
H8	**3.67**	**1.98**	**3.71**	**4.00**	**1.25**
H2′	0.32	0.02	0.09	0.13	0.01
H3′	0.32	0.02	0.09	0.13	0.01
H4′	0.39	0.01	0.15	0.24	0.13
H5′	0.38	0.01	0.15	0.24	0.13
H6′	0.45	0.09	0.34	0.39	0.27
H1″	0.32	0.02	0.09	0.08	0.04

[a] ΔT_1 is the variation of the relaxation time of each signal with respect to the corresponding one in free CQ (**1** in $CDCl_3$ and **2**−**5** in MeOD) [b] ^{1}H NMR signal was very broad and no accurate value of T1 could be measured for this proton.

The ^{1}H NMR signal for H2 in **4** was very broad, most likely due to quadrupolar relaxation arising from its proximity to the quinoline and ethylenediamine nitrogen atoms, and therefore no accurate value of T1 could be measured for this proton. In the case of the π-bonded derivative **5**, large ΔT_1 values are observed for all three protons at the carbocyclic ring (H8 ≫ H5 > H6), whereas in the heterocyclic ring H3 virtually does not shift, although H2 experiences a moderate shift; very small shifts are also observed for the aliphatic protons. These data are essentially consistent with the observed $\Delta\delta$ values for both ^{1}H and ^{13}C, indicating π-bonding of CQ to Ru through the carbocyclic ring.

In conclusion, the set of NMR experiments performed shows that ^{1}H ΔT_1 and ^{13}C $\Delta\delta$ values provide a much better indication than ^{1}H $\Delta\delta$ values for assigning the coordination site of a ligand with multiple possible binding modes, and this can be a valuable technique in those cases where X-ray structures are not available.

DFT Calculations. Additional support for our structural assignments has been obtained by the DFT-based computation of relative energies in the gas phase and in aqueous solution. The structures of **1**−**4** for isomers **A** and **B**, and of **5** in isomeric forms **C** and **D** were fully optimized in the gas phase. The polarizable continuum model[27] was employed to assess the relative energies of these structures in aqueous solution. Figure 1 shows the optimized structures for **1′** and **5** in their two isomeric forms **A** and **B**, and **C** and **D**, respectively (optimized structures and total energies for all other complexes can be found in the Supporting Information). Table 2 summarizes the relative energies (in kcal/mol) of the two possible CQ-binding modes for each complex in the gas phase and in aqueous solution. The energetic summary clearly demonstrates that in **1**−**4** Ru

(34) (a) The X-ray structure of $[Au(CQ)(PPh_3)]NO_3$ was recently mentioned in a poster presentation,[32b] and it confirms the assignment we made on the basis of NMR data when we first synthesized this compound in 1997.[17,] (b) Hitosugi-Levesque, M.; Tanski, J. M. InAbstracts of Papers, 236th ACS National Meeting, Philadelphia, PA, United States, August 17−21, 2008.

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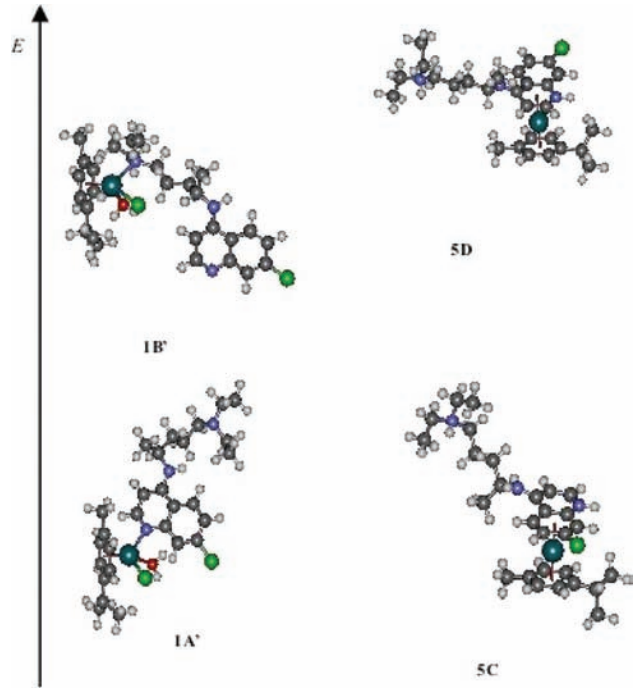


Figure 1. Optimized structures for **1′** and **5**.

Table 2. Energy Differences (in kcal/mol) between Isomeric Forms for Ru−CQ Complexes[a]

complex	ΔE (gas)	ΔE_{aq}
1	−3.08	−6.38
2	−4.59	−7.53
1′	−18.14	−4.34
2′	−18.22	−5.66
3	−16.44	−8.56
4	−33.26	−27.53
5	−22.34	−21.95

[a] $\Delta E = E_1 - E_2$; E_1 and E_2 are the total energies of the **A** and **B** isomers of **1**−**4**, respectively, and of isomers **C** and **D** for **5**.

coordination to the quinoline nitrogen atom (isomer **A**) is preferred over binding at the side chain nitrogen (isomer **B**). For the π-bonded derivative **5,** Ru coordination at the carbocyclic ring (**5C**) is evidently favored over the heterocyclic ring (**5D**), in good agreement with our conclusion based on $\Delta\delta$ and ΔT_1 NMR data.

In addition, the FTIR spectral signature of **1** measured in the 1500−1700 cm^{-1} region, compared with computed IR spectra for both isomers **1A** and **1B** is shown in Figure 2.

Experimental and computed frequencies, their relative IR intensities, and vibrational assignments based on the normal-mode analysis of computed harmonic force field are listed in Table 3. The computed spectra are systematically up shifted compared to experimental signals by about 3% (40−50 cm^{-1}), which is generally expected for computed vibrational bands because they are subject to systematic errors due to basis sets truncation, incomplete treatment of electron correlation, and a harmonic approximation. The errors are commonly corrected by applying empirical or semiempirical scaling factors; nevertheless, even without scaling of computed frequencies, definite assignments of experimental bands are easily achieved in this case.

The most intense band in the region measured at 1587 cm^{-1} is assigned to quinoline ring deformation with C═C

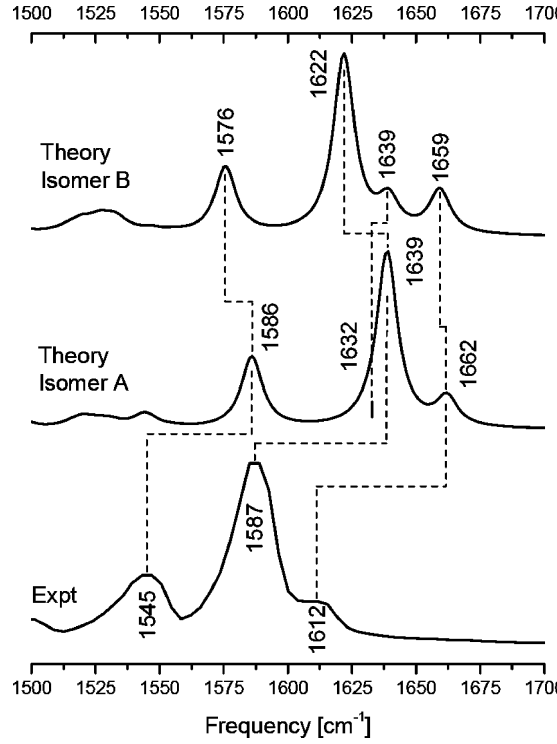


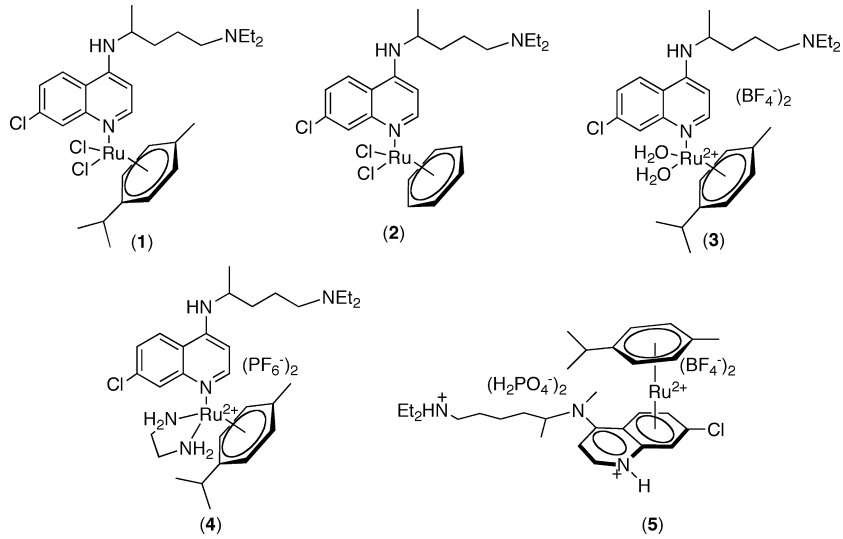
Figure 2. IR spectra for **1**. Spectra **A** and **B** are DFT simulated for the two isomers of **1**. The bottom spectrum is an experimental measurement.

Table 3. Experimental and Calculated Frequencies (cm^{-1}) and Relative Intensities (%) of IR Bands for Model Compounds

1 (exptl)	isomer **1A** (calcd)	isomer **1B** (calcd)	assignment
1542(m)	1584(44%)	1576(37%)	NH rock
	1631(1%)	1639(18%)	NH rock + *C*═N stretch (N-ring)
1587(s)	1637(100%)	1622(100%)	C═C stretch + C−H bend (N-ring)
1614(m)	1661(16%)	1659(25%)	C═C stretch + CH bend (C-ring)

stretch and C−H bending mostly localized on the heterocyclic ring and is computed at 1637 cm^{-1} for isomer **A** and at 1622 cm^{-1} for isomer **B**. The other two bands observed in the region are assigned to an NH rock (at 1542 cm^{-1}) and another quinoline ring deformation involving C═C stretch and C−H bending, localized on the carbocyclic ring (at 1614 cm^{-1}). These two bands are also successfully predicted for both isomers of **1**. One additional band in this region, predicted by DFT calculations at 1631 cm^{-1} for isomer **A** and at 1639 cm^{-1} for isomer **B**, is of particular interest for our discussion of spectral assignment and isomer recognition. The band is assigned to an NH rock mixed with the *C*═N stretch of the quinoline ring and is predicted to be moderately IR intense and detectable only for isomer **B** while down shifted by about 8 cm^{-1} and with virtually undetectable intensity for isomer **A**. Thus, the IR spectra of **1** supported by computational analysis, and assignments of vibrational bands are sugestive again that isomer **A**, in which CQ binds to Ru through the nitrogen atom of the quinoline moiety, is the most favorable form of the complex. A similar study was conducted for **2** (Supporting Information), and the corresponding analysis is also in agreement with Ru coordination taking place at the quinoline nitrogen. In summary, DFT results are consistent with the conclusions extracted from NMR spectra as described above.

Chart 3. Structural Assignments for **1**−**5**.



It is important to note that although each of the individual techniques used in this study may leave some ambiguities in the assignment of a bonding mode for some of the complexes, perusal of the combined experimental and theoretical data at hand leads to a very coherent picture that allows us to confidently propose the structures depicted in Chart 3 for the new Ru−CQ complexes, even if their amorphous nature in the solid state precluded X-ray diffraction studies. For 16 electron Ru(η^6-arene)X_2 fragments, CQ-coordination through the quinoline ring nitrogen atom is preferred, whereas the highly unsaturated Ru(η^6-arene) moiety binds to CQ through an η^6-interaction at the carbocyclic ring; this is the first example of η^6-bonding of CQ to a transition metal but a similar preference has been previously observed in Ru^{II} complexes of quinoline.[37] Another point of interest is that our proposed assignments correspond to molecular structures in solution, which are the ones responsible for the biological properties studied.

Biological Properties. The new Ru−CQ complexes described in this article follow a molecular design that displays a number of interesting features indicative of a potential therapeutic value. The metal is present in the +2 oxidation state, which is believed to be the active form for antiparasitic and antitumor action of compounds of this metal.[2−12] The arene ring serves to stabilize the metal center, to increase the lipophilicity of CQ, and to modulate the electronic properties of the complex; one of the chloride ligands is very labile, resulting in rapid aquation, a reaction also associated with biological activity. The second chloride ligand is inert in aqueous solution but it can be removed readily by silver salts to generate dicationic species; this allows us to assess the influence of the overall charge on the biological properties. The coordinated chloroquine is expected to take part in antimalarial and possibly antitumor mechanisms. The molecular modification of chloroquine together with the increased lipophilicity may result in a lower malaria parasite resistance, whereas the close association of chloroquine and Ru^{II} may lead to an enhancement of the antitumor activity. Also, importantly from a practical point of view, all the complexes are soluble in water or buffer, and the resulting solutions are stable for extended periods of time, even in the presence of air.

Antimalarial Activity. The activity of **1**, **2**, **3**, and **5** was evaluated in vitro against four CQ-sensitive strains (FcB1, 3D7, PFB, and F32) and three CQ-resistant strains (W2, Dd2, and K1) of *P. falciparum*. As can be observed from the data collected in Table 4, all of the compounds tested exhibit activity against the malaria parasites. In the case of the CQ-sensitive strains FcB1, PFB, and F32, the activities displayed by the Ru−CQ complexes are in general somewhat lower than the one observed for the standard drug CQDP, except in the case of the 3D7 strain, for which the metal derivatives are about twice as active as CQDP. There are no significant differences in the activities of the various new Ru−CQ complexes.

More importantly, in the case of the CQ-resistant parasites Dd2, K1, and W2, the potency of all complexes are consistently higher than that of CQDP, with the single exception of **5** being of slightly lower activity than CQDP against the highly resistant W2 strain. The highest activities were observed for the dicationic N-bonded **3**, reaching values around 5 times better than CQDP for the Dd2 and K1 strains and 2.4 times better for W2.

It is thus clear that the combination of Ru^{II} and chloroquine in a single molecule does produce an enhancement of the activity *against resistant strains* of the parasite, demonstrating the validity of our concept in the search for novel CQ-derived antimalarial drugs capable of overcoming resistance. We have previously observed a similar enhanced activity against CQ-resistant *P. falciparum* for the compound $[RuCl_2(CQ)]_2$[15] and proposed a mechanism of action involving heme aggregation inhibition within the acidic digestive vacuole of the parasite as the target, with the lowered resistance being due predominantly to an increase in the lipophilicity of the drug.[16] Further work is currently in

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Table 4. Antimalarial Activity of New Ru−CQ Complexes Against *P. falciparum*; IC_{50} = 50% Inhibitory Concentration (nM)[a]

strain	independent experiments (n)	IC_{50} (nM) (Relative Activity)[b] CQDP	**1**	**2**	**3**	**5**
FcB1	$n = 1$	40	50	75	65−70	65−70
	$n = 1$	45.6 ± 4.5	**(0.8)**	**(0.5)**	**(0.6)**	**(0.6)**
	(triplicate)		120.0 ± 4.0	81.6 ± 1.2	96.5 ± 6.0	
			(0.3)	**(0.5)**	**(0.4)**	
PFB	$n = 1$	58.3 ± 8.5	109.0 ± 15.1	79.0 ± 4.9	122.6 ± 15.2	
	(triplicate)		**(0.5)**	**(0.7)**	**(0.5)**	
F32	$n = 1$	8.2 ± 1.3	11.5 ± 1.8	13.2 ± 0.2	22.5 ± 0.4	
	(triplicate)		**(0.7)**	**(0.6)**	**(0.4)**	
3D7	$n = 3$	39.5 ± 7.0	19.6 ± 3.4	17.9 ± 1.5	19.5 ± 2.1	19.3 ± 2.8
	(triplicates)		**(2.0)**	**(2.2)**	**(2.0)**	**(2.0)**
Dd2	$n = 3$	1184 ± 188	483 ± 110	442 ± 30	234 ± 41	557 ± 49
	(triplicates)		**(2.5)**	**(2.7)**	**(5.0)**	**(2.1)**
K1	$n = 3$	1883 ± 165	600 ± 87	508 ± 84	353 ± 61	529 ± 97
	(triplicates)		**(3.1)**	**(3.7)**	**(5.3)**	**(3.6)**
W2	$n = 3$	2155	1667	1619	906	2549
			(1.3)	**(1.3)**	**(2.4)**	(0.8)

[a] For details, see "Antimalarial Activity Measurements" in the Experimental Section. [b] Relative activity = IC_{50} (CQDP)/ IC_{50} (complex).

Table 5. Antitumor Activity of New Ru−CQ Complexes; IC_{50} (μM)[a]

complex	HCT-116p53$^{+/+}$	HCT-116p53$^{-/-}$	LS141 (sarcoma)
1	20	20	8
5	35	35	18

[a] For details of Colorimetric Cell Proliferation assay, see the Experimental Section.

progress with the aim of understanding the mechanisms of action of the new metal−chloroquine derivatives and developing further series of compounds with higher antimalarial potency.

Antitumor Activity. 1 and **5** were selected as representative of the N-bonded and π-bonded CQ structures for testing against two HCT-116 colon cancer cell lines that differ in their p53 status (p53$^{+/+}$ and p53$^{-/-}$) and a liposarcoma cell line LS141 that is also wild-type for p53; the data are collected in Table 5. After 72 h of drug exposure, the two HCT-116 cell lines were more sensitive to **1** than to **5** with IC_{50} values of 20 and 35 μM, respectively. These values are comparable to what has been observed for other ruthenium complexes,[23,38] and they did not differ by p53 status. A similar behavior was observed by some of us in the case of $RuCl_2(KTZ)_2$, which is active against C8161, WM164 melanoma, and HT-29 human colon (IC_{50} 25 μM) and induces caspase-3 activation irrespective of p53 status.[12] This is important in connection with drug resistance, which may be associated with p53 mutational status.

More interestingly, treatment of the dedifferentiated liposarcoma cell line revealed a striking sensitivity to both complexes, especially to **1** (IC_{50} 8 μM). Consistent with the colon cancer cell lines, LS141 was more sensitive to **1** than to **5** (IC_{50} 18 μM); however, **5** was more active against LS141 than to either of the colon cancer cell lines. *Because there are no effective therapies in the treatment of liposarcoma, this could have potential application to the treatment of patients with this disease*. The basis for the increased sensitivity of LS141 to both complexes and the increased sensitivity of LS141 to **1** as opposed to **5** is unclear at this stage. The LS141 cell line is wild-type for p53 and also has amplification of MDM2. Whether the increased sensitivity of LS141 to the ruthenium complexes is due to differences in genetic background or to pharmacological differences (i.e., drug accumulation) will be the subject of further investigation.

Conclusion

We have synthesized a series of new Ru complexes containing arene and chloroquine ligands and characterized them by a combination of NMR, FTIR, and DFT methods; chloroquine binds to ruthenium preferentially through the quinoline nitrogen atom, whereas chloroquine diphosphate is π-bonded through the carbocyclic ring. **1**, **2**, **3**, and **5** display activity against *P. falciparum*; importantly, the potency of all complexes against CQ-resistant parasites is consistently higher than that of CQDP, demonstrating that the Ru−CQ combination is able to affect the parasite's resistance mechanisms. **1** and **5** also inhibit the growth of colon cancer cells, independently of the p53 status and of liposarcoma tumor cell lines. There appears to be a difference in sensitivity according to tumor subtype, with the liposarcoma cell line showing increased sensitivity to these complexes, especially **1**, for which an IC50 value of 8 μM was observed; this is significant because this type of tumor does not respond to currently employed chemotherapies.

Acknowledgment. We thank the NIH for financial support (to R.A.S.-D.) through Grant # 1S06 GM 076168-04.

Supporting Information Available: ^1H and ^{13}C $\Delta\delta$ data for all complexes. Computed total energies for **1**−**4** (isomers **A** and **B**) and **5** (isomers **C** and **D**). Optimized structures for all new complexes. IR spectra (calculated and experimental for **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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