Journal of Inorganic Biochemistry 104 (2010) 967–977

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Arene–Ru(II)–chloroquine complexes interact with DNA, induce apoptosis on human lymphoid cell lines and display low toxicity to normal mammalian cells

Alberto Martínez [a], Chandima S.K. Rajapakse [a], Roberto A. Sánchez-Delgado [a,*], Armando Varela-Ramirez [b], Carolina Lema [b], Renato J. Aguilera [b]

[a] Chemistry Department, Brooklyn College and The Graduate Center, The City University of New York, 2900 Bedford Avenue, Brooklyn, NY 11210, United States
[b] Department of Biological Sciences, Biosciences Research Building, University of Texas at El Paso, 500 West University Ave., El Paso, TX 79968, United States

ARTICLE INFO

Article history:
Received 18 February 2010
Received in revised form 6 May 2010
Accepted 10 May 2010
Available online 15 May 2010

Keywords:
Anticancer drug
Apoptosis
Binding affinity
Toxicity
DNA binding
Ruthenium complexes

ABSTRACT

The complexes [Ru(η^6-*p*-cymene)(CQ)Cl_2] (**1**), [Ru(η^6-benzene)(CQ)Cl_2] (**2**), [Ru(η^6-*p*-cymene)(CQ)$(H_2O)_2$]$[BF_4]_2$ (**3**), [Ru(η^6-*p*-cymene)(en)(CQ)]$[PF_6]_2$ (**4**), [Ru(η^6-*p*-cymene)(η^6-CQDP)]$[BF_4]_2$ (**5**) (CQ = chloroquine base; CQDP = chloroquine diphosphate; en = ethylenediamine) interact with DNA to a comparable extent to that of CQ and in analogous intercalative manner with no evidence for any direct contribution of the metal, as shown by spectrophotometric and fluorimetric titrations, thermal denaturation measurements, circular dichroism spectroscopy and electrophoresis mobility shift assays. Complexes **1**–**5** induced cytotoxicity in Jurkat and SUP-T1 cancer cells primarily via apoptosis. Despite the similarities in the DNA binding behavior of complexes **1**–**5** with those of CQ the antitumor properties of the metal drugs do not correlate with those of CQ, indicating that DNA is not the principal target in the mechanism of cytotoxicity of these compounds. Importantly, the Ru–CQ complexes are generally less toxic toward normal mouse splenocytes and human foreskin fibroblast cells than the standard antimalarial drug CQDP and therefore this type of compound shows promise for drug development.

1. Introduction

Cisplatin, carboplatin and oxaliplatin are widely used as first line treatments for cancer; nevertheless, toxicity and resistance limit their clinical efficacy [1,2]. *trans*-Pt(II) [3–6], octahedral Pt(IV) [7], or polynuclear Pt complexes [8–10] have been proposed as alternatives but they have not yet reached clinical use. Ruthenium complexes are emerging as promising candidates for novel cancer therapies for several reasons: This metal has several oxidation states accessible under physiological conditions [11]; Ru(II) and Ru(III) preferentially form octahedral compounds that interact with macromolecules in a different manner from those of platinum. More importantly, ruthenium complexes are able to mimic iron in binding biologically relevant molecules such as albumin and transferrin and as a consequence their toxicity is much lower than that of platinum therapies [12]. Two Ru-based drugs are in clinical development: (ImH)[*trans*-$RuCl_4$(DMSO)(Im)] (Im = imidazole) (NAMI-A) is effective against lung metastases [13–17]; although this compound interacts with DNA *in vitro* [15] such binding may not contribute to the anticancer mechanism. On the other hand, (IndH)[trans-$RuCl_4(Ind)_2$]) (Ind = indazole) (KP1019) is active against colon carcinomas [18–24] and DNA has been proposed as a possible important target [23].

Organometallic compounds are another source of anticancer drugs with $(\eta^5\text{-}C_5H_5)_2TiCl_2$ as the first of such species in clinical trials [25]. [Ru(η^6-arene)(X)(Y–Z)] complexes (where Y–Z is a chelating ligand, and X is monoanionic ligand) are highly cytotoxic against human ovarian tumor cells [26–29] and they are thought to act through covalent Ru–DNA interactions [30,31]. Related compounds incorporating the 1,3,5-triaza-7-phosphaadamantane (PTA) ligand, e.g. [Ru(η^6-*p*-cymene)(PTA)Cl_2] (RAPTA-C), have shown activity against metastases and although their mechanism of action has not been established, a pH dependent interaction with DNA may be a key component [32].

We have been investigating ruthenium complexes of known drugs as potential new chemotherapeutic agents for different applications [33]. The complexes $Ru(KTZ)_2Cl_2$ and $Ru(CTZ)_2Cl_2$ (KTZ = ketoconazole; CTZ = clotrimazole) display enhanced activity against *Trypanosoma cruzi*, the causative agent of Chagas' disease, and lowered toxicity to normal mammalian cells, in relation to the free ligands. These properties are due to a dual mechanism involving Ru-DNA binding and sterol biosynthesis inhibition by KTZ or CTZ [34–36]. $Ru(KTZ)_2Cl_2$ also induces cytotoxicity and apoptosis-associated caspase-3 activation in several cancer cell lines with IC_{50} values ~25 μM; this complex is more effective than cisplatin at inducing PARP fragmentation and proapoptotic Bak expression, suggesting that Ru(II) and Pt(II) complexes act through alternative signaling pathways [37]. We have also designed new antimalarial agents derived from Ru complexes of chloroquine (CQ), which was the drug of choice for decades until parasite resistance became widespread [38–41]; binding CQ to Ru results in an enhancement

* Corresponding author. Tel.: +1 718 951 5000x2827; fax: +1 718 951 4607.
E-mail address: RSdelgado@brooklyn.cuny.edu (R.A. Sánchez-Delgado).

0162-0134/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.jinorgbio.2010.05.002

of the efficacy against resistant strains of *Plasmodium falciparum*. In a recent paper we described the complexes Ru(η^6-arene)(CQ)L_2 (**1**–**4**) and [Ru(η^6-*p*-cymene)(η^6-CQDP)][BF_4]$_2$ (**5**) (Fig. 1) (CQDP = chloroquine diphosphate), which are up to 5 times more active than CQ against resistant strains of *Plasmodium falciparum* [42], due to an adequate combination of lipophilicity, basicity and structural features [43]. These arene–Ru–CQ complexes are also of great interest in cancer research, since (i) they are structurally related to the [Ru(η^6-arene)(X)(Y–Z)] and [Ru(η^6-*p*-cymene)(PTA)Cl_2] active compounds mentioned above; and (ii) CQ itself has some anticancer activity, induces preventive effects and enhances the effectiveness of other anticancer drugs [44–49]. It was thus reasonable to expect that a combination of both motifs in a single molecule would lead to enhanced antitumor activity. Indeed, [Ru(η^6-*p*-cymene)Cl_2(CQ)] (**1**) and [Ru(η^6-*p*-cymene)(η^6-CQDP)][BF_4]$_2$ (**5**) were found to be active against HCT-116 colon cancer cell lines and against a dedifferentiated liposarcoma cell line LS141 (IC_{50} 8 μM for complex **1**), for which there are no chemotherapies [42].

As far as the mechanism of action is concerned, DNA can be considered a potential target for the Ru–CQ compounds because (i) CQ binds to DNA through intercalation [50–56], along with electrostatic interactions of the ionic side chain with the DNA phosphate groups [57–59]; and (ii) the related compounds [Ru(η^6-arene)(X)(Y–Z)] exert their antitumor action through covalent binding of Ru to DNA. It was thus important to investigate the interactions of our compounds with DNA and to establish any possible relevance to their antitumor behavior.

Here we show that complexes **1**–**5** are cytotoxic to Jurkat human T lymphocyte leukemia and SUP-T1 lymphoma cells with preferential induction of apoptosis; we also describe the interactions of these compounds with DNA in relation to the antitumor behavior and we show that the complexes display low toxicity toward normal mouse splenocytes and human foreskin fibroblasts. The combined results suggest that this family of compounds is promising for drug development.

2. Experimental

2.1. General

Calf Thymus (CT) DNA, pBR322 plasmid DNA, buffers and solvents were purchased from Sigma-Aldrich. Solvents were purified by use of a PureSolv purification unit from Innovative Technology, Inc.; all other chemicals were used as received. Spectrophotometric studies and thermal denaturation experiments were performed on an Agilent 8453 diode-array spectrophotometer equipped with a HP 89090 Peltier temperature control accessory. Steady-state fluorescence measurements were carried out using a Spex Fluorolog Tau 2 fluorimeter (SPEX-Horiba Instruments, Inc., New Jersey) equipped with a thermostated cuvette holder. CD spectra were taken in a Chirascan CD Spectrometer also equipped with a thermostated cuvette holder. The complex [Ru(η^6-*p*-cymene)(en)Cl][PF_6] (**6**) (en = ethylenediamine) was prepared following the procedure described by Crabtree et al., using NH_4PF_6 instead of $NaBPh_4$ [60].

The synthesis of complexes **1**–**5** was previously described by us in detail; the characterization was achieved by a combination of 1D and 2D ^{1}H and ^{13}C NMR spectroscopy, combined with FTIR measurements and DFT (DFT = density functional theory) calculations [42]; in short: [Ru(η^6-arene)Cl_2(CQ)] (**1**, **2**). [Ru(η^6-arene)Cl_2]$_2$ (1 mmol) and CQ (2 mmol) were stirred in an appropriate solvent (30 mL) under N_2 at room temperature. The resulting mixture was evaporated to dryness and the product was redissolved and purified by filtration or crystallization. [Ru(η^6-*p*-cymene)(H_2O)$_2$(CQ)][BF_4]$_2$ (**3**). [Ru(η^6-*p*-cymene) Cl_2]$_2$ (0.65 mmol) and AgBF4 (2.61 mmol) were stirred in acetone (40 mL) at 55 °C under N_2. The solution was filtered through celite; CQ (1.31 mmol) was added and the mixture was allowed to react at 55 °C for 20 h. The resulting solution was dried under vacuum to obtain a brown solid, which was purified by recrystallization. [Ru(η^6-*p*-cymene)(en)(CQ)][PF_6]$_2$ (**4**). CQ (0.21 mmol) and $AgPF_6$ (0.21 mmol) were added to a solution of [Ru(η^6-*p*-cymene)Cl(en)][PF_6] (0.21 mmol) in methanol. The mixture was allowed to react for 20 h under N_2 at room temperature, then evaporated and the product was extracted with acetone and precipitated with diethyl ether. [Ru(η^6-*p*-cymene)(η^6-CQDP)][BF_4]$_2$ (**5**). [Ru(η^6-*p*-cymene)Cl_2]$_2$ (0.49 mmol) was dissolved in water; $AgBF_4$ (1.96 mmol) and chloroquine diphosphate (0.98 mmol) were added under N_2. The mixture was stirred for 20 h at 55 °C and then filtered through celite. The solvent was evaporated and the final product was dried under vacuum. Once isolated, the Ru(II) complexes were found to be very stable as solids and as aqueous solutions. Compounds **1** and **2** rapidly (<1 min) exchange one of the chloride ligands by water to form [Ru(η^6-arene)(CQ)(H_2O)Cl]Cl (**1′** and **2′**), as shown by electrical

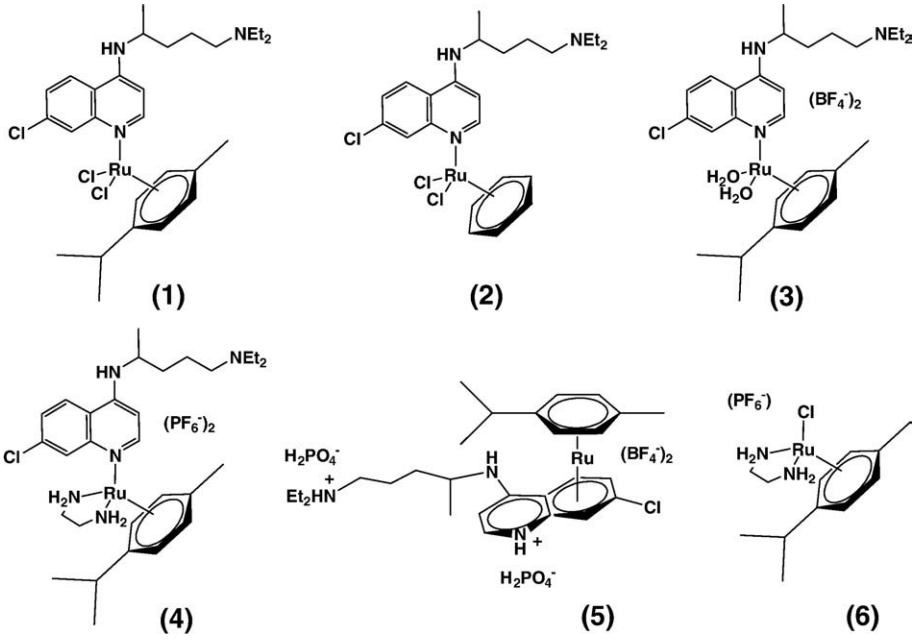

Fig. 1. Structures of the complexes object of this study: [Ru(η^6-*p*-cymene)(CQ)Cl_2] (**1**); [Ru(η^6-benzene)(CQ)Cl_2] (**2**); [Ru(η^6-*p*-cymene)(CQ)(H_2O)$_2$][BF_4]$_2$ (**3**); [Ru(η^6-arene)(en)(CQ)][PF_6]$_2$ (**4**); [Ru(η^6-*p*-cymene)(η^6-CQDP)][BF_4]$_2$ (**5**); [Ru(η^6-*p*-cymene)(en)Cl][PF_6] (**6**).